Probing Through-Bond Connectivities and Through-Space Distances in Solids by Magic-Angle-Spinning Nuclear Magnetic Resonance

M. Baldus, R. J. Iulucci, and B. H. Meier*

Contribution from the NSR-Center for Molecular Structure, Design and Synthesis, Laboratory of Physical Chemistry, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Received July 1, 1996®

Abstract: The application of a new solid-state NMR technique, total through-bond correlation spectroscopy, and of an improved scheme for total through-space correlation spectroscopy to structure determination in organic solids is explored. Both experimental techniques are employed under high-resolution conditions using magic-angle sample spinning (MAS). The results from a test sample (calcium acetate monohydrate) show that through-space and through-bond connectivities can be probed separately. The information contained in the two experiments is complementary and the combination of both results can lead to improved structure determination schemes in biomolecular and material-science applications.

Introduction

The methodology for the determination of the molecular geometry and topology by solid-state NMR spectroscopy is rapidly emerging and complete structures of biomolecules determined by solid-state NMR have been published (e.g. ref 1). Much of this work is motivated by the enormous success of NMR structure determination of dissolved molecules, in particular of biological macromolecules. Most commonly, the structure from liquid samples is obtained by combining experimental results that probe the through-bond connectivity (e.g. COSY4 or TOCSY6,7) with results that probe the through-space distances (e.g. NOESY7). These pulse schemes rely on polarization or coherence transfer during the mixing period of a two-dimensional experiment. The work presented here is concerned with the possibility of applying similar schemes in the solid state. In the solid, further structural and assignment information can be extracted if the anisotropic properties of the chemical shielding or the dipolar interaction are exploited. Here we will concentrate on “isotropic” spectra.

In the solid state, much progress has recently been reported toward structure determination using the through-space interactions. In contrast to liquid-state spectroscopy, the proton spectrum of solids is usually insufficiently resolved and we focus here on 13C spectroscopy in uniformly enriched samples. Extension to 15N, 31P, and other spin V


spinning (MAS). Then, polarization transfer caused by the dipole interaction can be observed. One approach to do so is a rapid reorientation of the axis of sample rotation away from the magic angle for the duration of the mixing time.8–10 A second approach leaves the sample-spinning axis at the magic angle. For a specific spin pair the dipolar interaction can be reintroduced by setting the rotor frequency equal to or equal to a submultiple of the isotropic chemical-shift difference (rotational resonance).11–13 Total through-space correlation spectroscopy (TOSSY) reintroduces simultaneously all dipolar connectivities in a single two-dimensional experiment. TOSSY employs, during the mixing time, a pulse sequence (usually rotor synchronized) that leads to broadband polarization transfer under MAS conditions.14–25

Recently, we have proposed an alternative total correlation experiment that monitors through-bond connectivities.26 In this


The mixing consisted of an integer number $m$ basic RIL-ZQT cycles, each of which is repeated $n$ times per rotor cycle. Because the mixing Hamiltonian is isotropic, the desired component of the polarization after $t_1$ must be selected either by a z-filter or by an appropriate difference experiment. For all experiments described, the MAS spinning speed was set to 4.53 kHz. The measurements were performed on a 400-MHz Chemagnetics Infinity spectrometer using a standard 3.2 mm double-resonance MAS probehead tuned to minimize asymmetric phase transients.

The J-angle concept introduces the idea of a two-stage scheme where proton homonuclear Lee–Goldburg decoupling eliminates the $^1$H–$^1$H interactions and the remaining (and now heterogeneous) $^1$H–$^{13}$C interactions are removed by a combination of sample rotation and $^{13}$C radio frequency irradiation.

For the TOSSY experiment, we require (i) that the cross-peak intensity reflects the intermolecular distance and is not considerably influenced by spectral quantities, e.g. the chemical shifts, and (ii) that the polarization transfer takes place through zero-quantum transitions that conserve the sum polarization of the spin system. Then, the polarization exchange is, for spin systems with an extended coupling network, described by a kinetic matrix. The RIL-ZQT scheme (see Figure 1a) has been optimized for that purpose and shall be used in the following for TOSSY experiments. In the delta-pulse limit and to zero-order average Hamiltonian approximation, the RIL dipolar mixing Hamiltonian in circular frequency units is given by:

$$H = \sum_{k=1}^{2} \frac{3M_0q_yq_y^H}{8\pi^2} \sin(2\theta) \sin(\phi) (3I_kJ_{1k} - \tilde{T}_{1k}\tilde{T}_{k})$$

The polar angles $\theta$ and $\phi$ orient the internuclear vector $\vec{r}_{ij}$ with respect to the MAS rotation axis. All dipolar-coupled spins in the sample contribute to the sum in (1) and their resonance lines are connected by cross peaks in a TOSSY spectrum. The RIL cycle must be applied rotor synchronously but no particular ratio (see Figure 1a) between carbon radio frequency field strength $\omega_c$ and MAS frequency $\omega_\text{mas}$ must be obeyed as long as $\omega_c$ is approximately an order of magnitude larger than $\omega_\text{mas}$. This condition can typically be fulfilled in practice.

In the TOBSY experiment, shown in Figure 1b, one attempts to completely suppress the dipolar interaction as well as the chemical-shielding terms. Then one can observe polarization transfer under the scalar mixing Hamiltonian (again to zero-order average Hamiltonian theory):

$$H = 2\pi \sum_{m-n} J_{mn} \tilde{t}_m\tilde{t}_n$$

The $J_{mn}$ denote the scalar coupling constants among the bonded spins $(m,n)$. In practice, the TOBSY experiment leads to cross peaks between all spins that are coupled through scalar couplings (directly or relayed) that exceed a certain threshold. Spectral resolution of the $J$ coupling in the $^{13}$C spectrum is not necessary. The basic pulse cycle, shown in Figure 1b, must be applied $k$ times per rotor cycle to obtain the Hamiltonian of eq 2. A ratio of $k = 4$ leads to an average Hamiltonian that contains no dipolar terms but still contributions from the chemical-shift anisotropy. We have found by numerical simulations that, for $^{13}$C spectroscopy, the field strength on the observe channel should be of the order of 100 kHz to efficiently suppress the isotropic and anisotropic chemical-shift contributions. The information contents of the TOBSY spectrum corresponds to that of the TOCSY spectrum of a dissolved compound.

Finally, it should be mentioned that, in principle, the TOSSY spectra can also contain cross-peak intensity caused by $J$ interactions. In practice this is, however, not problematic because of the larger size (usually by an order of magnitude) of the dipolar interaction.

Structure Determination in Organic Solids

Figure 2. TOBSY spectrum of the doubly-labeled monohydrate of calcium acetate. Only the carboxylic and aliphatic regions of the total 2D spectrum are shown, the rest of the spectrum is empty. Cross peaks are found within pairs of carboxylic and aliphatic resonances indicating intramolecular transfer only. 512 \( t_1 \) experiments were performed with 16 scans each. The mixing time was set to 20 ms. Further experimental parameters are given in the legend of Figure 1. Contour levels are shown for constant intervalls between 2 and 15% of the maximal signal intensity. The signals marked by an asterisk are assigned to a second crystal form present as a contamination.

Figure 3. TOSSY spectrum of the same sample as used in Figure 2. Intramolecular as well as intermolecular cross peaks are detected. The mixing time was 20 ms. Further experimental parameters and contour level settings are chosen as described in the legends to Figures 1 and 2.

Figure 4. Curve a: intensity of the TOBSY cross peak \( S(\text{cross}) \) between peak 4 and 4' (see Figure 2) as a function of the mixing time. The intensity is normalized to the total signal intensity of one spin pair \( S(\text{total}) \) which amounts to \( \frac{1}{8} \) of the total spectral intensity. An oscillatory transfer behavior is observed. Curve b: TOSSY buildup for the same pair using the pulse scheme of Figure 1. Curves c and d: intermolecular TOSSY cross-peak buildup between pair [2,3] and pair [2,4], respectively.

The oscillatory behavior suggests that the cross peaks are actually caused by \( J \) interactions and not by insufficiently suppressed dipolar interactions. Possible unwanted (dipolar) contributions are expected to grow monotonous as a function of the mixing time. An upper limit to the unwanted cross-peak intensity can be estimated from the first minimum of the oscillation in Figure 4. Possible “false” spin-diffusion cross peaks must be smaller, at 17 ms mixing, than 0.1. The same value is reached, by \( J \) transfer, after approximately 1.5 ms.

Experimental Section

TOBSY and TOSSY experiments were performed on a powder sample of doubly \( ^{13}\text{C} \) labeled calcium acetate monohydrate (Ca(CH\(_3\)\(_3\)-COO\(_2\)\(_2\)-H\(_2\)O). This compound crystallizes in a triclinic structure with four molecules per unit cell.\(^{32}\) For a control sample with \( ^{13}\text{C} \) at natural isotopic abundance, the four crystallographically distinct carboxylic carbons were fully resolved while the four methyl resonances show partial overlap. For the fully labeled sample, the spectral resolution is slightly lower (due to unresolved \( J \) couplings and further broadening mechanisms) and the methyl resonances are not resolved anymore. Two of the four resonances in the carboxylic region of the spectrum are fully resolved in the TOBSY and TOSSY spectra of Figures 2 and 3, respectively, while two of the resonances show partial overlap. In addition, we found an additional smaller resonance at 181 ppm, most probably due to contamination with another crystal form (marked by an asterisk in the figures).

Discussion

In the TOBSY spectrum (Figure 2) intramolecular cross peaks for each of the resolved crystallographically non-equivalent molecules are found while no intermolecular cross peaks are detected even at a mixing time of 20 ms (see Figure 2). The intensity of the TOBSY cross peaks as a function of the mixing time, for the cross peak involving the carboxylic resonance labeled by 4 (see Figure 2), is shown in Figure 4 (curve a). An almost undamped oscillatory time-dependence is observed. The same behavior is found for all four cross peaks. The observed oscillation frequencies, \( 60 \pm 10 \text{ Hz} \) for all bonded pairs, are in good agreement with the expected size for the \( ^{13}\text{C}^{-13}\text{C} \) \( J \) coupling that, in liquid phase, was determined to be \( 52 \text{ Hz}^{33} \).


\(^{33}\) Papavoine, T., personal communication.
The results presented show that through-space and through-bond connectivities can be probed separately in high-resolution solid-state NMR. The complementary information contained in the two experiments has been the basis of many successful applications to structure determination in liquid-state spectroscopy and we hope that the combined application of these two schemes to solid samples will be fruitful for biomolecular and material-science studies.

For applications to more complex spin systems further issues like sensitivity and spectral resolution become relevant. In this respect the TOBSY experiment is expected to perform very similarly to the TOSSY experiment which has been successfully applied in relatively complex systems. The maximum distances and the extend of the through-bond connectivities that can be probed by TOSSY and TOBSY are presently limited to about 5 Å and three-bond connectivity, respectively. This limitation is caused by the decay of the sum polarization of the $^{13}$C (or $^{15}$N) nuclei due to imperfect proton decoupling. Further investigations to improve this aspect of the experiment are presently under way.

Acknowledgment. We thank Jan M.A. Aelen for his help with preparing the labeled sample. Technical support by Jan van Os, Hans Janssen, and Gerda Nachtegaal and financial support by SON is gratefully acknowledged. Scientific discussions with Prof. Richard R. Ernst, Dr. Arno P. M. Krentgens, and Rene Verel and comments by two referees have been helpful.

JA9622259