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High Magnetic Field for Enhanced Proton Resolution in High-Speed CP/MAS Heteronuclear $^{1}H-^{13}C$ Dipolar-Correlation Spectroscopy

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It is generally accepted that heteronuclear-correlation spectroscopy in solids requires the application of multiple-pulse techniques to achieve sufficient line narrowing in the proton dimension. We now demonstrate that increasing the magnetic field strength improves the resolution dramatically, to such an extent that $^{1}H-^{13}C$ correlations and proton shifts can be directly obtained from 2-D spectra collected without any homonuclear decoupling scheme during t_1 evolution.

Recently, it has been shown that dipolar-correlation spectroscopy is an indispensable tool for the assignment of resonances and improvement of resolution in modern multidimensional high-resolution solid-state NMR (*1, 2*). For instance, structure refinement of large solid-type biological preparations using 2-D homonuclear ^{13}C dipolar-correlation spectroscopy is rapidly forthcoming, and has already yielded the first characterization of a solid-state structure, at the molecular level, of an intact biological system that is inaccessible to X-ray or solution NMR approaches (*3, 4*). In contrast, high-resolution heteronuclear ($^{1}H-^{13}C$) dipolar-correlation spectroscopy has been notoriously difficult until now, as it relies thus far on the application of elaborate multiple-pulse homonuclear decoupling sequences in combination with the MAS to suppress the strong dipolar interactions between the abundant protons and to achieve adequate resolution in the proton dimension (*5–9*). In general, the CRAMPS and related techniques require careful and often time-consuming adjustment of several instrumental parameters, and a very stable high-power RF performance (*10–15*). The use of trains of thousands of high-power pulses in combination with a high duty cycle represents a considerable drawback with respect to the lifetime of both probe and (pre)amplifers. In addition, a formal requirement for the application of CRAMPS techniques is that the cycle time of the CRAMPS sequence should be small compared to the rotor cycle time, which effectively limits the spinning speed and the efficacy of the MAS averaging. Finally, changing between samples with different dielectric properties and varying the temperature of the probe during the CP/MAS experiments affects the characteristics of the RF circuitry considerably. In particular, the application of tuned-up multiple-pulse sequences for high-resolution solid-state NMR spectroscopy of biological systems is quite impractical, if not entirely impossible.

In the description of the NMR response of strongly dipolar coupled protons in solids, the relatively small ^{1}H chemical-shift dispersion is usually neglected. This is definitely correct at lower fields, and for moderately high spinning speeds. In the strong dipolar limit, the proton levels are nearly degenerate, and the T_2-type relaxation responsible for the large proton linewidth easily proceeds throughout the rigid dipolar lattice. It is obvious that this approximation must break down when the magnetic field is sufficiently strong. It has been proposed that the dipolar interactions are effectively truncated by the chemical shift in high fields, yielding narrower ^{1}H lines (*16*). This very same mechanism is in fact also responsible for the remarkably narrow lines in the 2-D $^{13}C-^{13}C$ dipolar-correlation spectra of uniformly or multispin enriched biomolecules (*2–4*).

The purpose of the present Communication is to demonstrate the effectiveness of increasingly high fields, in combination with rapid spinning, for enhancing the proton resolution in heteronuclear $^{1}H-^{13}C$ dipolar-correlation spectroscopy. To this end, the most straightforward and very simple CP/WISE technique is used (Fig. 1), which does not comprise any elaborate proton line-narrowing methods (*17*). Following a 90° pulse on the protons, a time increment (t_1) before the CP allows the observation of the proton evolution with detection through the carbons. Since this technique requires virtually no parameter adjustment, it is much easier to implement and more robust than the multiple-pulse techniques. In addition, CP/WISE does not require extremely high RF power or long multiple-pulse trains.

The sequence depicted in Fig. 1 is optimized for use in high-resolution 2-D correlation spectroscopy in high fields with fast spinning. At high MAS frequencies, the Hartmann–Hahn matching and corresponding efficiency of CP magnetization transfer is very sensitive to RF power instabilities. To restore a broader matching profile, we used a ramped-amplitude CP sequence (RAMP-CP) (*18*). At the highest field strength, TPPM proton decoupling was used during the

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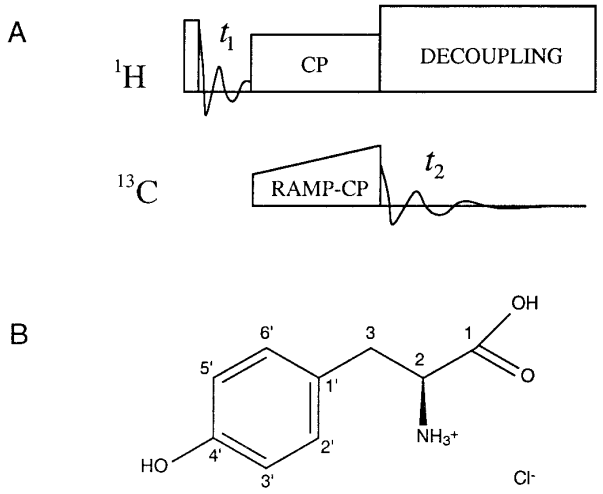

FIG. 1. CP/WISE pulse scheme used for the heteronuclear dipolar-correlation experiments (A) and chemical structure and IUPAC numbering scheme for the L-tyrosine·HCl salt (B).

^{13}C detection period t_2 (*19*). This is necessary since the TPPM provides a considerable improvement of ^{13}C resolution at higher fields. To avoid homonuclear coherence-transfer processes in both proton and uniformly labeled carbon spin reservoirs during CP, and to guarantee that each carbon will effectively receive its magnetization only from immediate protons, the RAMP-CP mixing times can be kept short, typically 50–200 μs. Finally, phase-sensitive detection in the ^{1}H dimension was simulated by varying the initial ^{1}H 90° pulse in a TPPI scheme (*20*).

Figure 2 shows the heteronuclear-correlation spectra of [U-^{13}C] L-tyrosine·HCl (Cambridge Isotopes), recorded at two different higher magnetic field strengths, 9.4 T (Fig. 2A) and 14.1 T (Fig. 2B). These data were obtained with an MSL 400 and a DMX600 spectrometer equipped with 4 mm MAS probes (Bruker, Karlsruhe, Germany). The spinning speed for the measurements at 9.4 T was 14.5 kHz, while the 14.1 T data sets were obtained with 15.0 kHz MAS. The RAMP-CP mixing times were fixed at 100 μs for both experiments. The phase-modulation angle for the TPPM decoupling was set to 20°, and the flip pulse length of 8 μs was adjusted to yield optimal ^{13}C resolution. Prior to Fourier transformation, a sine-squared apodization in the proton dimension was applied in the t_1 domain, with a Lorentzian broadening of 12 Hz in t_2. The data in Fig. 2 were plotted with linearly spaced contour levels. The digital resolution in the proton dimension is 390 and 234 Hz, with t_1 acquisition times of 1.280 and 2.132 ms, in 9.4 and 14.1 T, respectively. This is small compared to the proton linewidths.

It is clear from the two data sets shown in Fig. 2 that the overall resolution, in both dimensions, is already quite good at the moderately high field of 9.4 T and is considerably improved when the field strength is further increased to 14.1

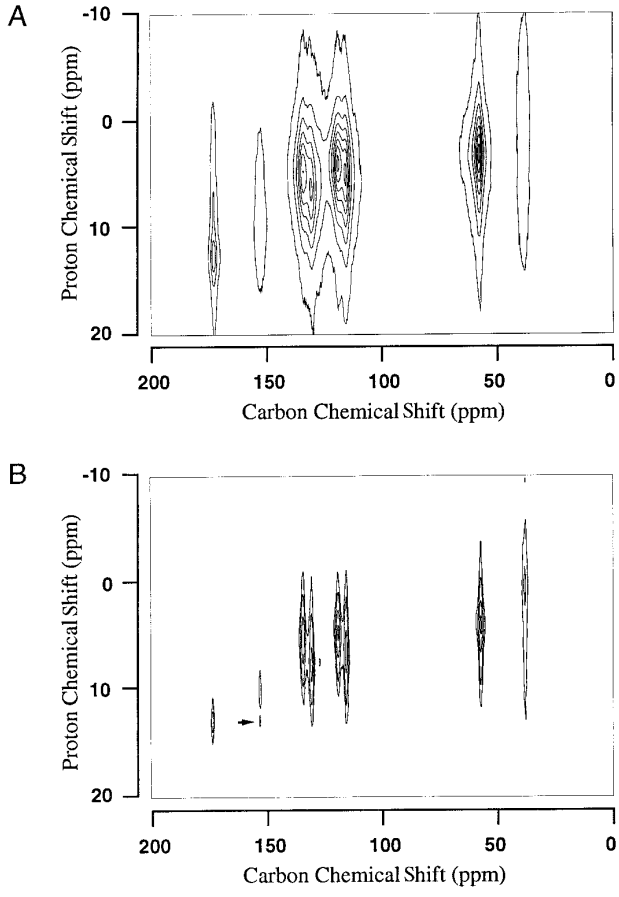

FIG. 2. Heteronuclear (^{1}H–^{13}C) dipolar-correlation spectra of [U-^{13}C] L-Tyr·HCl obtained with the pulse sequence of Fig. 1a in magnetic fields of 9.4 (A) and 14.1 T (B).

T. In particular, the proton chemical shifts are best resolved in the highest field (Fig. 2B), and an assignment of proton signals is easily achieved. The proton shifts in a field of 14.1 T are listed in Table 1. The carbons that resonate at 125.9,

TABLE 1
Solid-State Proton Shifts and Linewidths

Position	σ_i^a (ppm)	Linewidth (ppm) 9.4 T	Linewidth (ppm) 14.1 T
3-H	1	20	11
2-H	4.0	7.5	3.7
3′-H, 5′-H	4.7	6.3	3.5
	6.6	9	5.7
1′[b]	7	10	6
2′-H, 6′-H	5.2	7.5	4.5
	7.8	10	5
4′-OH	10.2	8.8	3.3
1-OH	12.9	5	1.8

[a] In a magnetic field of 14.1 T.
[b] Proton signal correlated with C1′.

151.6, and 173.1 ppm (C1′, C4′, and C1, respectively) show only weak correlations with the protons. This can be easily understood since no protons are directly bonded to these three carbons, and the magnetization transfer through the weaker $^{1}H-^{13}C$ couplings during the 100 μs mixing time to the carbons C1′, C4′, and C1 will be considerably less than for the other carbons in the molecule. The ^{1}H shift of 13 ppm for the correlation with the 1-^{13}C is the characteristic downfield range of 10–20 ppm for hydrogen-bonding protons.

In order to compare the resolution in the proton dimension in the two spectra of Fig. 2 in more detail, we extracted from the two-dimensional spectra vertical slices representing separate proton signals. The linewidths for these responses were measured from the data and are given in Table 1 for the two different fields. The improvement of the overall resolution with increasing field strength is obviously more than the factor 1.5 due to the increase of chemical-shift dispersion only. The effective gain in resolution, expressed in terms of the linewidths in ppm, is different for the various protons. For instance, for 2-^{1}H signal resonating at 4.0 ppm and correlated with the signal of 2-^{13}C resonating at 55.9 ppm, the effective resolution is improved by a factor 2. A somewhat smaller, but still comparable, improvement is obtained for the aromatic protons, while for the protons with the largest chemical shifts, at 10 and 13 ppm, the line-narrowing effect is even more substantial as the apparent resolution is improved, almost by a factor 3. In particular, the linewidth of ~2 ppm for the carboxylic hydrogen in the highest magnetic field compares well with typical widths achieved with other line-narrowing procedures. On the other hand, the 3-^{1}H line, correlated with the carbon signla at 36.7 ppm (3-^{13}C), is quite broad. This is only methylene carbon in the tyrosine molecule, with two strongly coupled protons with virutally identical chemical shifts.

Initially only the hydrogen-bonded proton resonating at 13 ppm appears to contribute to the cross-polarization transfer for the 1-^{3}C (Figs. 2B, 3). Similarly, the phenolic 4′-^{13}C appears to receive its polarization almost exclusively from the OH proton during the inital stages of the Hartmann–Hahn transfer (Fig. 2B). This is remarkable, since the two methylene protons, with signals at ~1 ppm, are also in proximity of the 1-^{13}C, while the two 3′,5′ aromatic protons are in fact closer to the 4′-^{13}C than the OH, but they do not contribute significantly to the initial CP transfer processes. This effect may prove quite useful in future studies of hydrogen-bonding characteristics of, e.g., biological samples and polymers, since it allows selective observation of signals from hydrogen-bonding protons.

The importance of the hydrogen-bonded protons in the initital stages of the CP transfer is also evident from the observation of a weak correlation of the 4′-^{13}C with the carboxylic proton at 13 ppm. This signal is only resolved in the data set obtained at 14.1 T and is indicated with an arrow in Fig. 2B. It is known from the crystal structure of L-tyrosine hydrochloride that the carboxylic proton forms an intermolecular hydrogen bond to a phenolic oxygen (*21*). The occurrence of an intermolecular correlation between 4′-^{13}C and the carboxylic proton resonance at 13 ppm means that the CP transfer of magnetization from the carboxyl proton along the hydrogen bond to the closest 4′-^{13}C in its phenolic hydrogen-bonding partner must be also quite efficient.

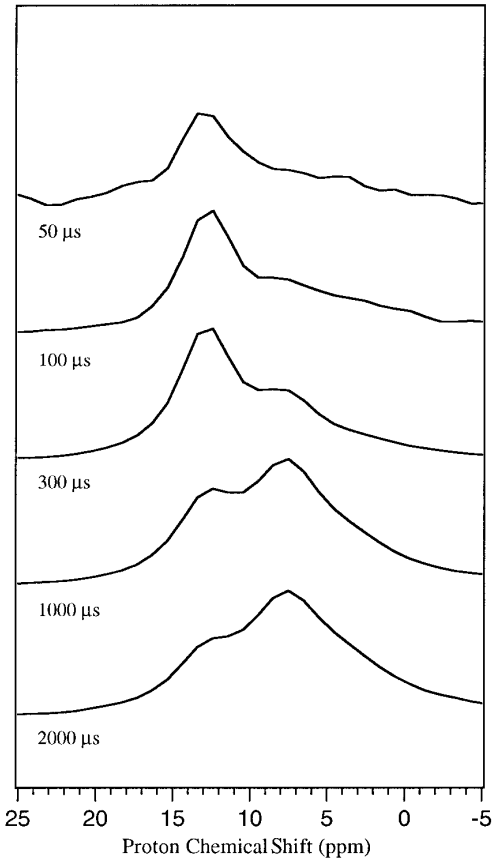

FIG. 3. ^{1}H responses associated with the 1-^{13}C at 173.1 ppm, extracted from a series of heteronuclear-correlation data sets, collected in a magnetic field of 9.4 T with the pulse sequence of Fig. 1A and different CP mixing times.

When the mixing time is longer, the coherence transfer during Hartmann–Hahn matching proceeds over larger distances, involving several protons and carbons. Hence, the resolution in the proton dimension is lost. This is nicely illustrated in Fig. 3, for the proton response correlated with the quaternary 1-^{13}C carbonyl signal. For longer mixing times, the dominant magnetization transfer originates from the aromatic region of the proton spectrum, yielding a broader correlation centered around ~7 ppm. Thus, varying the mixing time can provide information about the different possible pathways of magnetization transfer.

In conclusion, it is demonstrated for uniformly ^{13}C-enriched L-tyrosine HCl salt that (i) the resolution enhance-

ment in the dimension of the protons in a two-dimensional heteronuclear dipolar-correlation spectrum obtained with higher magnetic field strengths is sufficient to determine the proton chemical shifts directly from the 2-D experiment, (ii) it is possible to discriminate between contributions from different hydrogens in the immediate vicinity of a particular carbon, and (iii) hydrogen-bonding characteristics in the solid state can be determined. It is anticipated that further improvement can be obtained with higher magnetic field strengths, which will be crucial for future MAS structural investigations of large biological systems, for example, intrinsic membrance proteins and receptors.

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