Communications to the Editor

$^{13}C-^{13}C$ and $^{13}C-^{15}N$ Dipolar Correlation NMR of Uniformly Labeled Organic Solids for the Complete Assignment of Their ^{13}C and ^{15}N Signals: An Application to Adenosine

Toshimichi Fujiwara,*,† Kenji Sugase,†
Masatsune Kainosho,‡ Akira Ono,‡ Akira (Mei) Ono,‡ and
Hideo Akutsu*,†

Department of Bioengineering, Faculty of Engineering
Yokohama National University
Hodogaya-ku, Yokohama 240, Japan
Department of Chemistry, Tokyo Metropolitan University
1-1 Minamiohsawa, Hachioji, Tokyo 192-03, Japan

Received July 24, 1995

Nuclear spin interactions not averaged by isotropic motions can be exploited for the study of molecular structure in solids. Internuclear distances have been precisely measured from dipolar interactions.[1] However, such measurements require specific labeling of sites between which internuclear distances are determined. The preparation of such a specifically labeled sample is not trivial, and the number of spin−spin interactions measured for a single sample is limited.

Multidimensional isotropic-shift correlated NMR increases the number of spin connectivities obtainable in a single experiment by introducing additional frequency axes. Actually, heteronuclear multidimensional NMR for isotope-labeled molecules has been successful in structural determination of large molecules such as proteins and nucleic acids in solution.[2] This success is partially due to relatively easy preparation of uniformly labeled biomolecules. Recently, several methods have been proposed for broad-band magnetization transfer under magic angle spinning (MAS) conditions by spin−spin couplings in solids.[3-9] Theoretical background and an overview of the methods are given in ref 1. These methods have been used in 2-D isotropic-shift correlated NMR[7-11]for isotope-labeled molecules. We show that complete ^{13}C and ^{15}N assignments of organic solids can be performed by applying ^{13}C homonuclear and $^{13}C-^{15}N$ heteronuclear 2-D dipolar correlation NMR to the uniformly labeled organic molecules.

A ^{13}C homonuclear dipolar correlation experiment was carried out with the pulse sequence shown in Figure 1A. The two isotropic-shift evolution periods are connected by a ^{13}C homonuclear dipolar mixing period under the USEME sequence.[3] The ^{13}C correlation spectra obtained for labeled adenosine are shown in Figure 2. The mixing time for ^{13}C homonuclear magnetization transfer was 1.2 ms, which was chosen for the spin pairs connected only with direct covalent bonds to give rise to cross peaks. Since dipolar coupling constants between directly bonded nuclei are more than 4 times as large as those between nuclei not connected with direct covalent bonds, it is possible

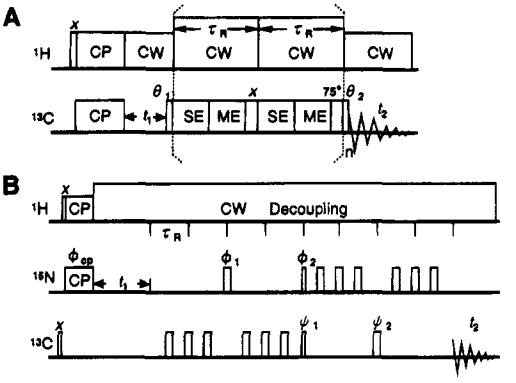

Figure 1. Pulse sequence for 2-D ^{13}C homonuclear dipolar correlation (A) and heteronuclear $^{13}C-^{15}N$ correlation (B) under MAS conditions. The narrow and wide pulses represent $\pi/2$ and π pulses, respectively. The initial magnetization is prepared by cross polarization (CP) from proton. In sequence A, magnetization is mixed among ^{13}C spins by homonuclear dipolar interactions recovered with pulse sequence USEME, in which spin echo (SE) and magic echo (ME) sequences are synchronized with the sample spinning period τ_R under continuous wave (CW) decoupling. The phase cycle is $\theta_1 = (-x, x)$, $\theta_2 = (x)$, and acquisition $= (x,-x)$. The phases for the $\pi/2(\theta_2)$ pulse and acquisition are further cycled according to CYCLOPS. The pulse phase θ_2 is shifted by 90° to generate hypercomplex data for phase sensitive detection. In sequence B, ^{15}N magnetization is transferred to ^{13}C with a TEDOR dipolar mixing sequence. The phase cycle is $\phi_{CP} = (x, -x)$, $\phi_1 = (x, -x, -x, x)$, $\phi_2 = (x)$, $\psi_1 = (x, -x)$, $\psi_2 = (x, x, -x, -x)$, and acquisition $= (x)$. The phases for the $\pi/2(\psi_1)$ and $\pi(\psi_2)$ pulses and acquisition are further cycled according to CYCLOPS. The constant-phase $\pi/2$ pulse immediately before CP cancels the initial ^{13}C magnetization. The CP phase is changed for the pure-phase detection in t_1. The phases of the carbon π pulses before $\pi/2(\psi_1)$ and those of the nitrogen π pulses following $\pi/2(\phi_2)$ are modulated so as to make a pulse train of $\pi_x\pi_x\pi_{-x}\pi_{-x}\pi_x\pi_x\pi_x$.

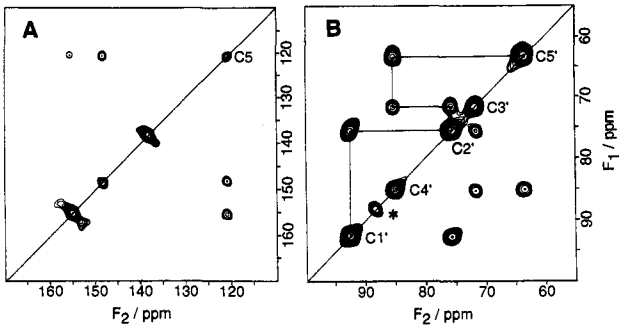

Figure 2. Purine (A) and ribose (B) regions of a 2-D ^{13}C homonuclear dipolar correlation spectrum. The carbon-13 experiment was carried out for 20 mg of [^{13}C,^{15}N]adenosine on an Otsuka Electronics CMX400 spectrometer operating at a ^{13}C resonance frequency of 100.1 MHz. Adenosine was uniformly enriched with ^{15}N and ^{13}C at 99% by the procedure in ref 12. The contact time for CP was 4 ms. The dipolar HOHAHA mixing (USEME) was carried out with a carbon rf amplitude of 50 kHz under 5-kHz MAS. The ^1H decoupling rf amplitude was 95 kHz during the mixing period. The recycle delay was 40 s. The number of scans for an FID was 8. A complex data matrix $128(t_1) \times 512(t_2)$ acquired was zero-filled to a 1024 × 1024 matrix. A Gaussian window function was applied. A spinning sideband is indicated by the asterisk (*). A sequential connectivity is shown by solid lines.

† Yokohama National University.
‡ Tokyo Metropolitan University.

(1) Bennett, A. E.; Griffin, R. G.; Vega, S. *NMR* **1994,** *33*, 1−77.
(2) Clore, G. M.; Gronenborn, A. M. *Science* **1991,** *252*, 1390−1398.
(3) Fujiwara, T.; Ramamoorthy, A.; Nagayama, K.; Hioka, K.; Fujito, T. *Chem. Phys. Lett.* **1993,** *212*, 81−84.
(4) Ramamoorthy, A.; Fujiwara, T.; Nagayama, K. *J. Magn. Reson., Ser. A* **1993,** *104*, 366−368.
(5) Hing, A. W.; Vega, S.; Schaefer, J. *J. Magn. Reson., Ser. A* **1993,** *103*, 151−162.
(6) Sodickson, D. K.; Levitt, M. L.; Vega, S.; Griffin, R. G. *J. Chem. Phys.* **1993,** *98*, 6742−6748.
(7) Baldus, M.; Tomaselli, M.; Meier, B. H.; Ernst, R. R. *Chem. Phys. Lett.* **1994,** *225*, 329−336.
(8) Sun, B. Q.; Costa, P. R.; Griffin, R. G. *J. Magn. Reson., Ser. A* **1995,** *112*, 191−198.
(9) Sun, B. Q.; Costa, P. R.; Kocisko. D.; Lansbury, P. T., Jr.; Griffin, R. G. *J. Chem. Phys.* **1995,** *102*, 702−708.

0002-7863/95/1517-11351$09.00/0

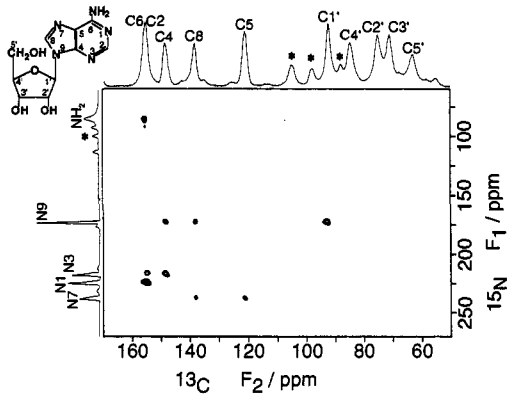

Figure 3. 2-D $^{13}C-^{15}N$ dipolar correlation spectrum and corresponding 1-D spectra obtained for 40 mg of [^{13}C,^{15}N]adenosine. The contact time for CP was 7.5 ms. The ^{13}C and ^{15}N rf amplitudes were 42 kHz. The ^{1}H rf amplitude was 52 kHz during the $^{13}C-^{15}N$ mixing and acquisition. The number of scans for an FID was 16. Chemical shifts for ^{13}C and ^{15}N are relative to hexamethylbenzene at 17.17 ppm and saturated $^{15}NH_4Cl$ at 27.34 ppm, respectively. The other experimental conditions were the same as those given in the caption below Figure 2. The atom numbering of adenosine and the signal assignments are also presented in the figure. The chemical shifts of the labeled adenosine are as follows: $C_{1'}$(92,6), $C_{2'}$(75.4), $C_{3'}$(71.5), $C_{4'}$(85.1) $C_{5'}$(63.2), N_1(223.9), C_2(154.8), N_3(217.2), C_4(148.5), C_5(120.8), C_6(155.6), N_7(237.7), C_8(138.1), N_9(172.5), and NH_2(85.2).

to find such a mixing time. Carbon connectivities in the sugar region are easily traced. Assuming that the signal at 92.6 ppm is the $C_{1'}$ resonance, we can automatically assign the other sugar carbon signals; the $C_{5'}$ signal should be a resonance at 63.2 ppm. Since three carbons C_4, C_5, and C_6 in the purine are linearly connected by covalent bonds, only the C_5 carbon has two cross peaks as shown in the spectrum.

A 2-D $^{13}C-^{15}N$ correlation spectrum (Figure 3) was obtained with the pulse sequence shown in Figure 1B. After the ^{15}N isotropic chemical shift evolution for t_1, the magnetization was transferred to carbon by the $^{13}C-^{15}N$ dipolar couplings recovered under the TEDOR sequence.[5,8] At the mixing time of 1.6 ms, large cross peaks appear only for pairs connected with $^{13}C-^{15}N$ covalent bonds. The cross peak between a nitrogen and a sugar carbon is due to magnetization transfer between $C_{1'}$ and N_9. Therefore, the assumed assignment of the $C_{1'}$ resonance in Figure 2B was verified by this experiment. The N_9 resonance couples with two adenine carbons, C_8 and C_4, as shown in the spectrum; and only C_4 of the two carbons has a cross peak in the ^{13}C homonuclear correlation spectrum (Figure 2A). A small chemical shift difference between C_2 and C_6 resonances can be measured from the C_2/N_3 and C_6/NH_2 cross peaks. Thus, all ^{13}C and ^{15}N signals of adenosine were identified unequivocally through one-bond dipolar couplings. The assignments are consistent with those of adenosine in solution.[13-15] So far, the ^{13}C and ^{15}N signals of nucleosides and nucleotides in solids[16,17] have been assigned with the aid of those in solution.

Complete assignments of ^{13}C and ^{15}N signals of organic molecules in solids are difficult in natural abundance, because there are no spin-spin couplings among ^{13}C and ^{15}N nuclei and chemical shifts of ^{1}H are usually not available owing to difficulties in the proton observation. Though the method presented in this work requires fully labeled molecules, it enables unambiguous assignments based on one-bond dipolar couplings recovered by the multipulses under MAS as long as signals are resolved in multidimensional spectra. Classification of carbons and nitrogens by the number of attached protons[17] complements this method. This method for the complete assignment shown in this paper will contribute to opening new horizons for structural analysis not only of biological molecules but also of organic solids and polymers.

Acknowledgment. This research was partially supported by the Grant-in-Aids for Specially Promoted Research (No. 05101004, H.A. and M.K.) and for Scientific Research of Priority Area (No. 06276102, T.F.) from the Ministry of Education and by a grant from Kanagawa Academy of Science and Technology (No. 94037, T.F.). M.K. acknowledges support from the special coordination fund of the Science and Technology Agency (Japan) and a grant of Human Frontier Science Programs (Strasbourg, France).

JA952453N

(10) Boender, G. J.; Raap, J.; Prytulla, S.; Oschkinat, H.; de Groot, H. J .M. *Chem. Phys. Lett.* **1995,** *237,* 502-508.
(11) Ono, A.; Tate, S.; Kainosho, M. In *Stable Isotope Applications in Biomolecular Structure and Mechanisms*; Trewhella, J., Cross, T. A., Unkefer, C. J., Eds.; Los Alamos National Laboratory: Los Alamos, 1994, pp 127-144.
(12) Jones, A. J.; Winkley, M. W.; Grant, D. M.; Robins, R. K. *Proc. Natl. Acad. Sci. U.S.A.* **1970,** *65,* 27-30.
(13) Mantsch, H. H.; Smith, I. C. P. *Biochem. Biophys Res. Commun.* **1972,** *46,* 808-815.
(14) Markowski, V.; Sullivan, G. R.; Roberts, J. D. *J. Am. Chem. Soc.* **1977,** *99,* 714-718.
(15) Chang, C.-J.; Dias, L. E.; Woolfenden, W. R.; Grant, D. M. *J. Org. Chem.* **1982,** *47,* 5318-5321.
(16) Cross, T. A.; Diverdi, J. A.; Opella, S. J. *J. Am. Chem. Soc.* **1982,** *104,* 1759-1761.