http://dx.doi.org/10.5012/bkcs.2013.34.10.2959

Deuterium Naturally Present in Solvent and Site-Specific Isotope Population of Deuterium-Enriched Solute

Ryeo Yun Hwang,[†,‡] Oc Hee Han,[†,‡,§,*] Juhee Lee,[†] and Eun Hee Kim[#]

[†]*Analysis Research Division, Daegu Center, Korea Basic Science Institute, Daegu 702-701, Korea*
[‡]*Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, Korea*
[§]*Department of Chemistry, Kyungpook National University, Daegu 702-701, Korea. [*]E-mail: ohhan@kbsi.re.kr*
[#]*Ochang Campus, Korea Basic Science Institute, Ochang 363-883, Korea*
Received July 8, 2013, Accepted July 14, 2013

As the concentration of aqueous CD_3OH solutions was decreased, the OD peaks in ^2H NMR spectra grew relative to the CD_3 peaks. Isotope impurity for OH groups of CD_3OH and deuterium naturally present in water contributed to the OD peaks. Using these peak area data, the site-specific isotope populations of isotope enriched chemicals were measured. In addition, the method using both ^1H and ^2H NMR spectroscopy was demonstrated with neat CD_3OH to measure the site-specific isotope populations. The results indicate that although it represents only ~0.015% of hydrogen isotopes, the deuterium naturally present in solvents cannot be ignored, especially when the concentration of deuterium-enriched solutes is varied. Proton/deuteron exchange between methyl and methyl/hydroxyl groups was confirmed to be negligible, while that among hydroxyl groups was detectable.

Key Words : NMR spectroscopy, Deuterium, Isotopes, Alcohol, Proton exchange

Introduction

Isotope-enriched chemicals have alleviated signal overlaps by shifting mass-to-charge ratio in mass spectroscopy (MS)[1-4] or vibrational frequencies in infrared or Raman spectroscopy.[5-7] Likewise, nuclear magnetic resonance (NMR) spectra have been clarified by altering *J*-coupling splitting patterns and chemical shifts using isotope enrichment.[2,8-12] In addition, isotope enrichment has been employed to enhance spectral resolution,[11,13] to remove background signals[4,14] and to increase sensitivity in NMR spectroscopy.[13,15,16] Labeling the specific sites of molecules with isotopes has made it easier to examine chemical reaction mechanisms.[17-19] Proton-deuteron exchange has been used to distinguish the surface sites and inner sites of proteins[4,20] and inorganic materials.[21] Site-specific natural isotope fractionation has been measured to trace the geographical origin and production year of agricultural products, and to detect mixing and adulteration in food and beverages.[22,23] Kinetic isotope effects have been investigated to probe transition states during chemical reactions, especially with hydrogen/deuterium isotopes, by using their relatively large mass ratio.[4,17,24]

Deuterium is the most frequently used hydrogen isotope and is relatively cheap. The presence of naturally abundant deuterium (~0.015%)[25,26] in solvent is typically ignored in spectroscopic data interpretation. We show a case in which the presence of naturally abundant deuterium in solvent should be considered, especially when the concentration of deuterium-enriched chemicals is varied. In this work, solutions of various concentrations were used to measure site-specific isotope impurities in isotope-enriched samples and the natural abundance of the isotope. Aqueous CD_3OH solutions were used, since water and methanol have been extensively studied in terms of hydrogen bonding and proton transfer,[8,12,24,27,28] and methyl and hydroxyl groups have been investigated due to their ubiquity and functions in biological systems.[12,13,29-31]

Experimental

Aqueous CD_3OH solutions of 11 different concentrations of 4, 2, 0.4, 0.2, 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, and 0.001 M were prepared by diluting CD_3OH (99.8 atom% D, Sigma-Aldrich) with distilled water. To improve the accuracy of the solution concentration, both the volume and weight of the CD_3OH and the distilled water used to prepare the 4 M solution were measured. Likewise, the volume and weight were measured when solutions with lower concentrations were prepared by diluting the solutions of higher concentrations. For NMR experiments, 100-μL solutions, which were measured accurately by the difference of the weights of the rotor before and after sample filling, were placed in 4-mm outer-diameter zirconia rotors for a double-channel magic angle spinning (MAS) probe. The ^2H NMR spectra were acquired using a 9.4-T DSX NMR system (Bruker BioSpin GmbH, Germany) with a pulse length of 2 μs corresponding to a 30° flip angle, a 10-s pulse repetition delay time, a 12-kHz spectral width, and 4 dummy scans. The number of acquisitions was varied from 16 to 4096, depending on the CD_3OH concentration. The sample weights and the peak areas for each concentration are listed in Table S2 and correlated in Figure S1 in the Supporting Information. The chemical shift was calibrated by setting the CD_3 signal of the

0.1 M CD_3OH solution at 3.3 ppm. For some of the solutions, ^2H NMR spectra were also acquired with a cryogenic inverse probe in an 18.8-T Avance II NMR system (Bruker BioSpin GmbH, Germany) with the solutions placed in 5-mm outer-diameter NMR tubes to achieve higher spectral resolution and to confirm the results obtained at 9.4 T.

Results and Discussion

When the concentrations of aqueous CD_3OH solutions were reduced, the signal for deuterated methyl (CD_3) groups at 3.3 ppm in ^2H NMR spectra was decreased, as expected (Figure 1(a)). However, at the same time, the signal of deuterated hydroxyl (OD) peaks at 4.8 ppm grew relative to the CD_3 signal (Figure 1(b)). The OD signals can come from naturally abundant deuterium in water, and from the OD groups present in CD_3OH as isotope impurities. Proton-deuteron exchange between CD_3 and OH groups can also contribute to OD signals, but this is not expected to occur much due to the large pK_a values of methyl protons/deuterons in aqueous solutions.[32]

The population of naturally abundant deuterium in water as a form of OD would be increased when the amount of water relative to CD_3OH is increased in a solution. The relative peak areas obtained from experimental NMR data (Figure 2) were well fitted with the calculated data, assuming that naturally abundant deuterium in water and the OD groups present in CD_3OH as an impurity are the sources of the OD signal, and that there is no proton-deuteron exchange between CD_3 and OH groups. Two different fitting methods were used. The simple method (Method I) involved the use of 0.015% for the naturally abundant deuterium in water, and the isotope impurities (H for CD_3, D for OH) present in neat CD_3OH liquid were determined from the peak areas in ^1H and ^2H NMR spectra of neat CD_3OH liquid. The relative peak areas for CD_3 and OD groups calculated using these values for each CD_3OH solution were compared with those obtained experimentally. In Method II, in order to obtain the values of deuterium abundance in water and isotope impurity for CD_3 and OH sites in CD_3OH, these values were iteratively varied until the sum of the peak area differences between the experimental and the calculated CD_3OH spectra for all of the CD_3OH concentrations examined reached a minimum.

Method I was carried out as follows. The peak areas for OH and CH_3 in an ^1H NMR spectrum of neat CD_3OH liquid were denoted as P_{OH} and P_{CH3}, respectively, and those for OD and CD_3 in a ^2H NMR spectrum of neat CD_3OH liquid as P_{OD} and P_{CD3}, respectively. Then, $(P_{OH} + \alpha P_{OD}):(P_{CH3} + \alpha P_{CD3})$ should be 1:3 from the ratio of hydrogen/deuterium numbers of hydroxyl groups and methyl groups. The amount

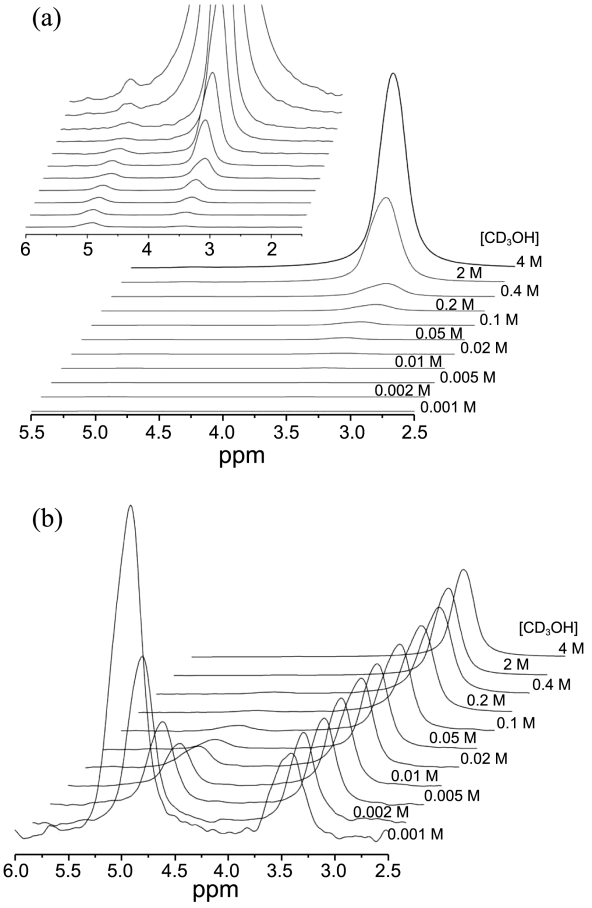

Figure 1. ^2H NMR spectra obtained at 9.4 T of aqueous CD_3OH solutions in various concentrations: (a) Spectra with spectral intensities calibrated with the number of acquisitions. Spectra expanded in their intensities are shown at the upper left. (b) Spectra with spectral intensities of CD_3 signals adjusted to have the same height.

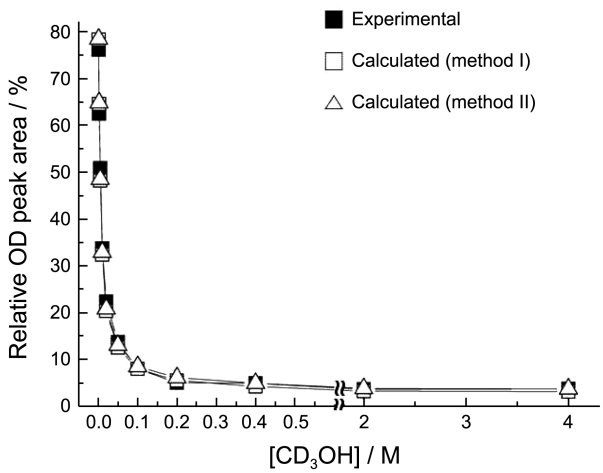

Figure 2. The plots of relative peak areas of OD groups (filled symbols) of ^2H NMR spectra of aqueous CD_3OH solutions in various concentrations and calculated peak areas (unfilled symbols) obtained by two different methods (I and II). For method I, 0.015% for the naturally abundant deuterium in water and the isotope impurities (H for CD_3, D for OH) present in neat CD_3OH liquid determined from the peak areas in ^1H and ^2H NMR spectra of neat CD_3OH liquid are used. In Method II, to obtain the values of deuterium abundance in water and the amounts of isotope impurity for CD_3 and OH sites in CD_3OH, these values are iteratively varied until the sum of the peak area differences between the experimental and the calculated spectra for all of the CD_3OH concentrations reaches a minimum.

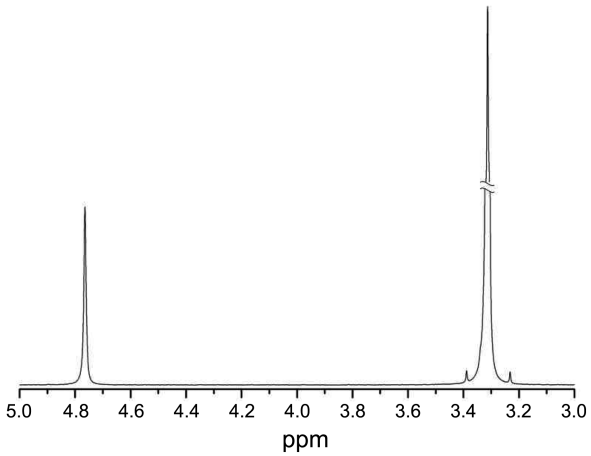

Figure 3. ^{2}H NMR spectrum of aqueous CD_3OH solution of 0.1 M obtained at 18.8 T.

of isotope impurity for CD_3 groups of CD_3OH can be defined as $I_{CD3} = P_{CH3}/(P_{CH3} + \alpha P_{CD3})$. Likewise, the amount of isotope impurity for OH groups of CD_3OH can be denoted as $I_{OH} = \alpha P_{OD}/(P_{OH} + \alpha P_{OD})$. Using the experimentally measured values of $P_{OH} = 0.960$, $P_{CH3} = 0.040$, $P_{OD} = 0.030$, and $P_{CD3} = 0.970$, α was calculated to be 3.227, for which $I_{CD3} = 0.013$ and $I_{OH} = 0.092$ were obtained. The relative signal intensities of CD_3 and OD groups can be estimated using the relationships of $3C_m\text{-}3C_mI_{CD3}$ and $2N_DC_w + C_mI_{OH}$, respectively, where C_m is the concentration of CD_3OH, C_w is the concentration of water, and N_D is the natural abundance of deuterium in water. The experimental and calculated data are compared in Figure 2 and Table S1. The regression coefficient (R^2) was 0.99502. For Method II, the sum of the peak area differences between the experimental and the calculated spectra for all of the CD_3OH concentrations was minimized by iterative calculation to obtain values for N_D, I_{CD3}, and I_{OH}. At most 2 unknown values can be obtained with two independent equations describing the data. Thus, N_D was fixed at 0.00015, and I_{CD3} and I_{OH} were obtained as 0.018 and 0.110, respectively. The larger R^2 value of 0.99684 than the value of 0.99502 obtained for Method I indicates that Method II is more reliable. Isotope impurity of ~1% for CD_3 groups is acceptable but ~10% isotope impurity for OH groups is unusually high.

The ^{2}H NMR spectra obtained with a high-resolution liquid-state NMR probe at higher magnetic field (18.8 T) clearly show a large center peak at 3.3 ppm for methyl deuterium bonded to ^{12}C and two small peaks, 21.7 Hz away from each other, at both sides of the large peak as shown in Figure 3. These two small peaks are from deuterium J_{CD}-coupled to the ^{13}C of CD_3, the total area of which corresponds to ~1% of the total peak area of CD_3 groups. If deuteron exchanges among $^{13}CD_3$ and $^{12}CD_3$ groups at fast rates, the CD_3 signals would merge to the ^{2}H signal of $^{12}CD_3$, which is at the gravimetric center of all the CD_3 signals. Likewise, if the deuteron/proton exchange is fast between methyl and hydroxyl groups, the CD_3 and OD peaks would merge. Thus, the split peaks for ^{2}H bonded to ^{13}C and the well-resolved CD_3 and OD peaks confirmed that the proton/deuteron exchange between methyl and methyl/hydroxyl groups, respectively, is negligible in the NMR time scale. On the other hand, single peaks for OD groups indicate fast proton/deuteron exchange among hydroxyl groups of water and methanol. If this exchange was slow in the NMR time scale, the OD and CD_3 peaks would be mutually split by J_{HD}-coupling (~1.7 Hz) between hydroxyl and methyl protons/deuterons.[33] Peak splitting due to J_{DD}-coupling for methanol can be practically ignored, since it is 6.51 times smaller than the corresponding J_{HD}-coupling of ~1.7 Hz.[34] The peak area ratios of methyl and hydroxyl groups did not differ from those obtained at 9.4 T.

Conclusion

We showed with aqueous CD_3OH solutions in various concentrations that the deuterium naturally present in water should be considered, even though the amount is not large (~0.015%), especially when the samples are prepared by diluting deuterated solutes in various amounts of water. This indicates that natural abundance of deuterium in other solvents than water should be equally considered. The method using both ^{1}H and ^{2}H NMR spectroscopy was demonstrated with neat CD_3OH to measure the site-specific isotope populations. Iterative calculation method was also used to minimize the sum of the differences of experimental and calculated ^{2}H peak areas of aqueous CD_3OH solutions. In addition, proton/deuteron exchange between methyl and methyl/hydroxyl groups was confirmed to be negligible, while that among hydroxyl groups was detectable. These results can be referenced for studying proton exchange between various functional groups and biochemical reaction mechanisms with isotope-enriched samples (especially with deuterium).

Acknowledgments. This work was supported by the KBSI grants (T33419) to O. H. Han and the National Research Foundation of Korea Grant funded by the Korean Ministry of Education, Science and Technology (2012 & 2013, University-Institute cooperation program). Dr. Seen Aae Chae, Sun Ha Kim, and Young Eun Lee at the KBSI are acknowledged for their technical support during the NMR experiments and Prof. Choel Ho Choi at KNU for stimulating discussion.

References

1. Zehl, M.; Rand, K. D.; Jensen, O. N.; Jørgensen, T. J. D. *J. Am. Chem. Soc.* **2008**, *130*, 17453-17459.
2. Tode, C.; Takeuchi, A.; Iwakawa, S.; Tatsumi, A.; Sugiura, M. *Chem. Pharm. Bull.* **2009**, *57*, 653-656.
3. Defoy, D.; Dansette, P. M.; Neugebauer, W.; Wagner, J. R.; Klarskov, K. *Chem. Res. Toxicol.* **2011**, *24*, 412-417.
4. Quint, P.; Ayala, I.; Busby, S. A.; Chalmers, M. J.; Griffin, P. R.; Rocca, J.; Nick, H. S.; Silverman, D. N. *Biochemistry* **2006**, *45*, 8209-8215.
5. Fanetti, S.; Ceppatelli, M.; Citroni, M.; Bini, R. *J. Phys. Chem. C* **2012**, *116*, 2108-2115.

6. Carmona, P.; Rodriguez-Casado, A.; Molina, M. *Anal. Bioanal. Chem.* **2009**, *393*, 1289-1295.
7. Garczarek, F.; Gerwert, K. *J. Am. Chem. Soc.* **2006**, *128*, 28-29.
8. Hanashima, S.; Kato, K.; Yamaguchi, Y. *Chem. Commun.* **2011**, *47*, 10800-10802.
9. Tolstoy, P. M.; Guo, J.; Koeppe, B.; Golubev, N. S.; Denisov, G. S.; Smirnov, S. N.; Limbach, H. H. *J. Phys. Chem. A* **2010**, *114*, 10775-10782.
10. Smirnov, S. N.; Golubev, N. S.; Denisov, G. S.; Benedict, H.; Schah-Mohammedi, P.; Limbach, H.-H. *J. Am. Chem. Soc.* **1996**, *118*, 4094-4101.
11. Agarwal, V.; Diehl, A.; Skrynnikov, N.; Reif, B. *J. Am. Chem. Soc.* **2006**, *128*, 12620-12621.
12. Dziembowska, T.; Hansen, P. E.; Rozwadowski, Z. *Prog. Nucl. Magn. Reson. Spectrosc.* **2004**, *45*, 1-29.
13. Ruschak, A. M.; Velyvis, A.; Kay, L. E. *J. Biomol. NMR* **2010**, *48*, 129-135.
14. Han, O. H.; Han, K. S.; Shin, C. W.; Lee, J.; Kim, S. S.; Um, M. S.; Joh, H.-I.; Kim, S.-K.; Ha, H. Y. *Angew. Chem. Int. Ed.* **2012**, *51*, 3842-3845.
15. Kim, I.; Han, O. H.; Chae, S. A.; Paik, Y.; Kwon, S. H.; Lee, K. S.; Sung, Y. E.; Kim, H. *Angew. Chem. Int. Ed.* **2011**, *50*, 2270-2274.
16. Paik, Y.; Kim, S. S.; Han, O. H. *Angew. Chem. Int. Ed.* **2008**, *47*, 94-96.
17. Limbach, H.-H. In *NMR*; Diehl, P., Fluck, E., Günther, H., Kosfeld, R., Seelig, J., Eds.; Springer-Verlag: Berlin., 1991; Vol. 23, pp 63-164.
18. Li, F.; Chen, Q.; Liu, C.-C.; Wu, Y.-H.; Liu, X.-P.; Yang, G.-F. *Appl. Magn. Reson.* **2012**, *42*, 169-177.
19. Scrivanti, A.; Beghetto, V.; Campagna, E.; Zanato, M.; Matteoli, U. *Organometallics* **1998**, *17*, 630-635.
20. Tang, Y.; Goger, M. J.; Raleigh, D. P. *Biochemistry* **2006**, *45*, 6940-6946.
21. Christy, A. A.; Egeberg, P. K. *Analyst.* **2005**, *130*, 738-744.
22. Košir, I. J.; Kocjančič, M.; Ogrinc, N.; Kidrič, J. *Anal. Chim. Acta.* **2001**, *429*, 195-206.
23. Cordella, C.; Moussa, I.; Martel, A.-C.; Sbirrazzuoli, N.; Lizzani-Cuvelier, L. *J. Agric. Food Chem.* **2002**, *50*, 1751-1764.
24. Gerritzen, D.; Limbach, H. H. *Ber. Bunsenges. Phys. Chem.* **1981**, *85*, 527-535.
25. Briggs, J. M.; Farnell, L. F.; Randall, E. W. *J. Chem. Soc., Chem. Commun.* **1973**, 70-71.
26. Martin, M.; Martin, G. In *NMR*; Diehl, P., Fluck, E., Günther, H., Kosfeld, R., Seelig, J., Eds.; Springer-Verlag: Berlin., 1991; Vol. 23, pp 1-61.
27. Souda, R.; Kawanowa, H.; Kondo, M.; Gotoh, Y. *J. Chem. Phys.* **2003**, *119*, 6194-6200.
28. Heller, J.; Elgabarty, H.; Zhuang, B. L.; Sebastiani, D.; Hinderberger, D. *J. Phys. Chem. B* **2010**, *114*, 7429-7438.
29. Otten, R.; Chu, B.; Krewulak, K. D.; Vogel, H. J.; Mulder, F. A. A. *J. Am. Chem. Soc.* **2010**, *132*, 2952-2960.
30. Ruschak, A. M.; Kay, L. E. *J. Biomol. NMR* **2010**, *46*, 75-87.
31. Chatterjee, J.; Rechenmacher, F.; Kessler, H. *Angew. Chem. Int. Ed.* **2013**, *52*, 254-269.
32. Grabowski, J. J. *Chem. Commun.* **1997**, 255-256.
33. Lewis, W. C.; Norcross, B. E. *J. Org. Chem.* **1965**, *30*, 2866-2867.
34. *NMR and the Periodic Table*; Harris, R. K., Mann, B. E., Eds.; Academic Press: London, 1978; p 9 and p 110.