Solid-State NMR Spectroscopy

International Edition: DOI: 10.1002/anie.201700488
German Edition: DOI: 10.1002/ange.201700488

Solid-State ^{17}O NMR Reveals Hydrogen-Bonding Energetics: Not All Low-Barrier Hydrogen Bonds Are Strong

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Abstract: *While NMR and IR spectroscopic signatures and structural characteristics of low-barrier hydrogen bond (LBHB) formation are well documented in the literature, direct measurement of the LBHB energy is difficult. Here, we show that solid-state ^{17}O NMR spectroscopy can provide unique information about the energy required to break a LBHB. Our solid-state ^{17}O NMR data show that the HB enthalpy of the O···H···N LBHB formed in crystalline nicotinic acid is only 7.7 ± 0.5 kcal mol^{-1}, suggesting that not all LBHBs are particularly strong.*

A low-barrier hydrogen bond (LBHB) refers to a HB in which the potential energy barrier for translational proton movement between the two heavy atoms is close to the zero-point energy.[1] The H atom in a LBHB is generally believed to delocalize between the two heavy atoms (e.g., O···H···O, O···H···N). As a result, the H atom in the LBHB does not form a standard covalent bond with neither the donor nor the acceptor heavy atom. A LBHB is commonly associated with a short distance between the two heavy atoms (e.g., $r_{OO} < 2.55$ and $r_{ON} < 2.70$ Å) and in the literature a LBHB is often automatically assumed to be a very strong HB (thus LBHB is often interchangeable with the term of short, strong HB, or SSHB). Although the existence of LBHBs in both gas and condensed phases has been firmly established, their role in enzyme catalysis has remained a controversy in the past 20 years.[2] The central issue is whether a LBHB is particularly strong as compared with a regular HB. While spectroscopic (mainly NMR and IR) signatures and structural characteristics of LBHBs are well documented in the literature, information about LBHB energetics is generally lacking, because it is rather difficult to directly measure it in the condensed phases. To date, assessments of the LBHB energy are largely based either on experimental determination of the free energy formation for HB (ΔG_f^{HB}) within a series of structurally related compounds,[3] or on quantum chemical computations.[4] In both these cases, LBHB energetics is often evaluated on a relative scale.

Now consider an O–H–N HB where the donor group is a carboxylic acid (AH) and the receptor is a pyridine derivative (B). It is generally accepted that the difference between the pK_a values of the AH and HB$^+$ functional groups, $\Delta pK_a = pK_a(AH) - pK_a(HB^+)$, is a major determinant for the type of HB formed. As illustrated in Figure 1, if

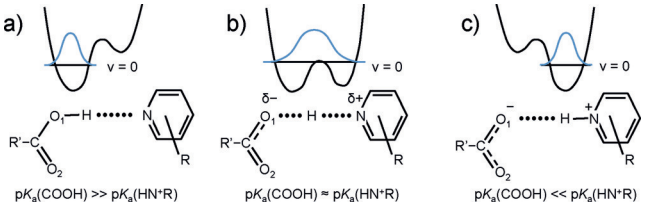

Figure 1. Characteristic 1D potential energy curves for the proton movement across a) regular O–H···N HBs, b) O···H···N LBHBs, and c) regular O···H–N HBs.

$\Delta pK_a \gg 0$, the H atom will be covalently bonded to the donor atom, that is O–H, and the potential energy curve would show a large asymmetry. In contrast, if $\Delta pK_a \ll 0$, the H atom would be covalently bonded to the acceptor atom, that is, H–N. However, when the two pK_a values become comparable ($\Delta pK_a \approx 0$), a LBHB is expected where the H atom is significantly delocalized.

The central hypothesis of the present work is that, in a LBHB of the type $O_2=C-O_1\cdots H\cdots N$, it may be possible for the carboxylate group to undergo a 180° flip motion causing an interchange of the chemical environments of the two oxygen atoms. As this carboxylate flip motion would break the HB, any information about the energy barrier of this motion will provide a direct measure of the energetics of the LBHB. To test this hypothesis, we performed a combined solid-state NMR and plane-wave DFT study on nicotinic acid (NA) crystals. NA was chosen for the following reasons. First, the crystal structure of NA shows that the NA molecules are linked by simple two-center OHN HBs to form a zig–zag chain along the *b*-axis,[5] as seen in Figure 2a. Second, as the carboxyl and pyridinium groups in NA exhibit very similar pK_a values (ca. 4.8–4.9),[6] NA would be an ideal LBHB model system where the donor and acceptor groups exhibit nearly matched proton affinities (i.e., $\Delta pK_a \approx 0$). Third, the O···H···N type of LBHBs is commonly found in enzymes.[7]

The first step in our study was to establish the nature of the HB in NA crystals. As previous crystal structures of NA are of only moderate quality,[5] we decided to collect higher quality diffraction data for a single crystal of NA at different

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Supporting information for this article can be found under: http://dx.doi.org/10.1002/anie.201700488.

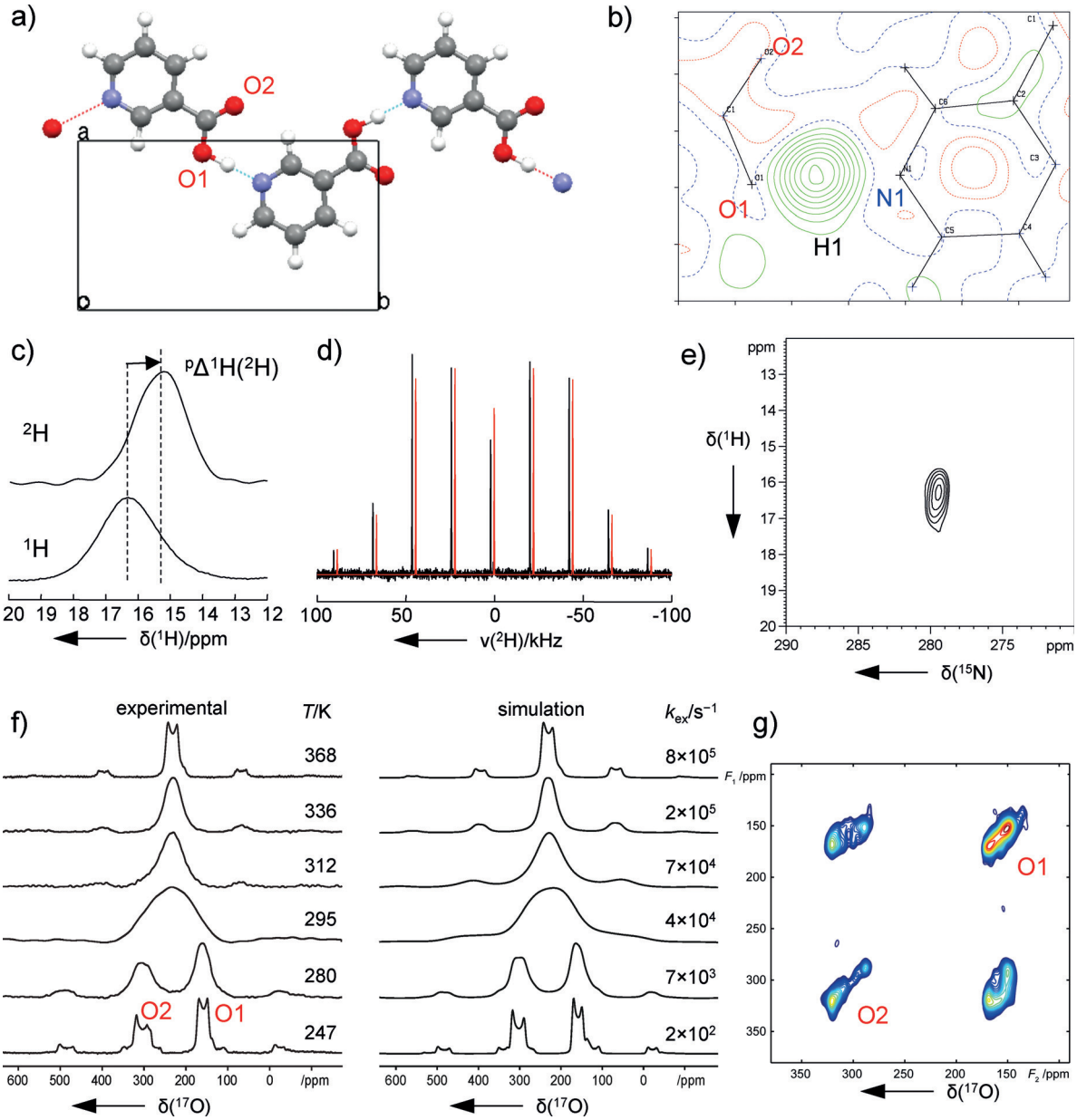

Figure 2. a) NA molecules form hydrogen bonded zig-zag ribbons along the crystallographic *b* axis. b) Electron density difference map of NA at 180 K. c) ^{1}H (60 kHz) and ^{2}H (22 kHz) MAS spectra of NA and [1-^{2}H]NA, respectively. d) Experimental (black trace) and simulated (red trace) ^{2}H MAS spectra of [1-^{2}H]NA. The simulated spectrum was deliberately shifted for easy comparison. e) ^{1}H-^{15}N 10 kHz CP/MAS HETCOR spectrum of [^{15}N]NA. f) Experimental (left) and simulated (right) variable temperature ^{17}O MAS spectra of [1,2-$^{17}O_2$]NA. g) 2D ^{17}O EXSY spectrum of [1,2-$^{17}O_2$]NA at 266 K. A mixing time of 100 ms was used.

temperatures. Figure 2b shows the electron density difference map of NA at 180 K. The O···N distance is 2.657 Å, which compares with the literature value, 2.661 Å, measured at 298 K.[5b] While the precise position of an H atom cannot be reliably determined from X-ray diffraction data, the difference map of Figure 2b nonetheless suggests an elongated O–H bond. For example, if we take the maximum position in the residual electron density map as the location of the H atom, the O–H distance would be 1.160 Å. In NA, the OHN HB is nearly linear, $\measuredangle$OHN = 177.6°. To further characterize the HB in NA, we performed a comprehensive solid-state NMR study. As seen from Figure 2c, the ^{1}H chemical shift for the H atom in question was found to be 16.2 ppm, which is within the range for LBHBs, ca. 16–20 ppm. The observed primary isotope shift in NA, $^{p}\Delta^{1}H(^{2}H) = \delta(^{1}H) - \delta(^{2}H) = +0.8$ ppm, is also typical of a LBHB.[1b] The ^{2}H quadrupolar coupling constant was 128 kHz, significantly smaller than that expected for a regular O–H covalent bond, about 200 kHz.[8] We note that the ^{1}H and ^{2}H NMR parameters found for NA are quite similar to those recently reported for the O···H···O LBHBs in dibenzoylmethane and curcumin.[9] We have also obtained the ^{15}N isotropic chemical shift (Figure 2e) and chemical shift tensor components for [^{15}N]NA (see Table 1 and Figure S1 in the Supporting Information). These ^{15}N NMR parameters are

Table 1: Experimental solid-state ^{1}H, ^{2}H, ^{14}N, ^{15}N, and ^{17}O NMR parameters obtained for NA, [1-^{2}H]NA, [^{15}N]NA, and [1,2-$^{17}O_2$]NA crystals.

Atom	δ_{iso} [ppm]	(δ_{11}, δ_{22}, δ_{33}) [ppm]	C_Q [MHz]	η_Q
^{1}H	16.2 ± 0.2	–	–	–
^{2}H	15.4 ± 0.2	–	0.128 ± 0.002	0.0
^{14}N	–	–	3.774[b]	0.284[b]
^{15}N	277.2 ± 0.2	(453, 421, −40)[a]	–	–
^{17}O, O1	179 ± 1	(339, 219, −21)[a]	7.0 ± 0.1	0.0
^{17}O, O2	329 ± 1	(521, 377, 89)[a]	8.3 ± 0.1	0.0

[a] The uncertainties in experimental chemical shift tensor components are: ^{15}N, ±2 ppm; ^{17}O, ±10 ppm. [b] From ref. [11].

in agreement with those observed for the LBHBs formed between carboxylic acids and pyridine derivatives reported by Limbach et al.[10] Finally, the ^{14}N quadrupolar coupling parameters ($C_Q = 3.774$ MHz and $\eta_Q = 0.284$) reported by Blinc et al.[11] from an NQR study of NA also suggest that the HB in NA is in between the pure O–H···N and O···H–N forms. Table 1 summarizes the solid-state NMR results for NA. These data strongly suggest the presence of an O···H···N type of LBHB in NA crystals. In addition, we attempted ^{1}H-^{15}N and ^{1}H-^{17}O dipolar recoupling experiments in the hope that we could directly measure the HB distances. However, the measured dipolar couplings appeared to be sensitive to temperature, suggesting the presence of considerably dynamics in NA, which is not unexpected as we discuss below. Nonetheless, at 213 K, our dipolar recoupling experiments indicate that the H atom is closer to the O atom than to the N atom; see Figure S2.

Figure 2 f shows the variable temperature (VT) ^{17}O MAS NMR spectra for [1,2-$^{17}O_2$]NA. At 247 K, the ^{17}O MAS spectrum consists of two signals, each displaying a characteristic second-order quadrupolar lineshape. An analysis of this low-temperature spectrum produced the ^{17}O NMR tensors listed in Table 1. As the temperature of the sample increases, the two ^{17}O NMR signals begin to broaden, merge into one broad signal (coalescence), which then sharpens upon further temperature increase. These spectral features are indicative of a two-site chemical exchange process between O1 and O2. While solid-state ^{17}O NMR spectroscopy has recently been used to study organic and biological molecules[12] and to assess HB energies in weak HBs,[13] this is the first time that this technique is employed for probing LBHB energetics. As seen from Figure 2 f, the experimental ^{17}O NMR MAS spectra can be very well reproduced by simulation that models a 180° flip motion for the carboxylate group. To further probe this chemical exchange process, we recorded a 2D EXSY ^{17}O MAS spectrum for [1,2-$^{17}O_2$]NA (Figure 2 g), from which cross peaks between O1 and O2 signals are clearly observed. The 1D spectral simulations also produced the exchange rate (k_{ex}) at each temperature. An Eyring analysis of the exchange rates yielded the following parameters for the carboxylate rotational barrier: $\Delta H^{\ddagger} = 11.5 \pm 0.5$ kcal mol^{-1} and $\Delta S^{\ddagger} \approx 0$ cal mol^{-1} K^{-1}; see Figure S3. Our DFT calculations indicate that, in the absence of HB, the rotational barrier of the carboxylate group in NA is 3.8 kcal mol^{-1}. Thus, breaking the LBHB in NA requires 7.7 ± 0.5 kcal mol^{-1}. This value can be interpreted as the LBHB enthalpy (ΔH^{HB}), because the HB interaction in the transition state of the carboxylate rotation is negligible. To independently assess the energy barrier for the carboxylate rotation in NA, we performed periodic DFT calculations using the CASTEP code.[14] The calculations yielded $\Delta H^{\ddagger} = 12.6$ kcal mol^{-1} (see Table S1) and a rotational barrier of 4.5 kcal mol^{-1} in the absence of HB. Thus the calculated ΔH^{HB} is 8.1 kcal mol^{-1}, which is in good agreement with the experimental value.

Before we further discuss the implication of the LBHB enthalpy determined for NA, we present more plane-wave DFT calculations for NA in order to gain further insights into the proton probability distribution across the LBHB. In this regard, we performed both adiabatic HB potential mapping and molecular dynamics (MD) simulations for the full periodic environment of NA. Figure 3 a shows the 1D

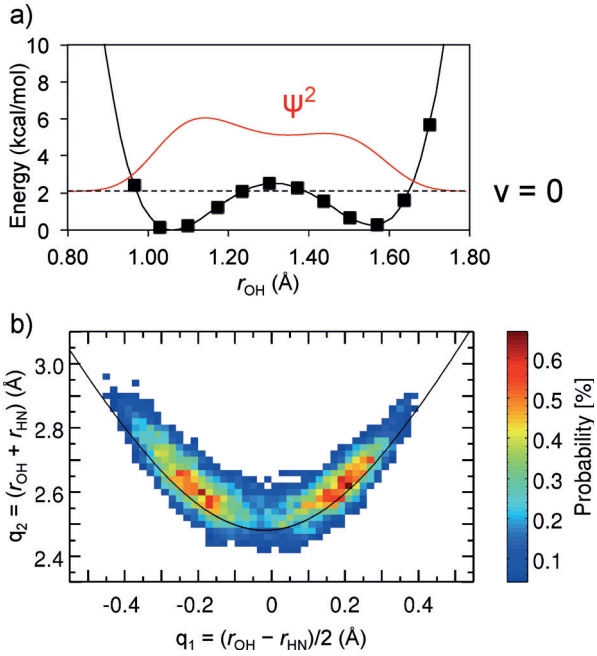

Figure 3. a) Adiabatic 1D HB potential and the 1D proton probability distribution (red line) for the vibrational ground state of NA. The black solid line is a polynomial fit of the data points (filled squares) obtained from CASTEP calculations. The dash line marks the zero-point energy. b) 2D proton probability distribution generated from plane-wave DFT MD simulation data. The solid line is calculated with the valence bond order model using the parameters given by Limbach et al.[10b] for describing OHN HBs.

adiabatic HB potential for proton movement in NA. The 1D nuclear wavefunction was obtained by solving the 1D Schrödinger equation using the Numerov method.[15] Since the barrier of proton movement is only 2.5 kcal mol^{-1} and the zero-point energy ($E_{v=0} = 2.0$ kcal mol^{-1}) is slightly below the barrier, a wide proton probability distribution is seen. In comparison, the MD simulations produced a smaller barrier (0.5 kcal mol^{-1}; see Figure S4). Recent studies have shown that, although the ab initio MD simulations treat the proton as a classic particle, they can still produce qualitative information about the proton probability distribution in the

cases of LBHBs.[9,16] Figure 3b shows the results of the plane-wave DFT MD simulations for NA as a 2D proton probability distribution in the q_1-q_2 space where $q_1 = (r_{OH} - r_{HN})/2$ and $q_2 = (r_{OH} + r_{HN})$ are the natural hydrogen bond coordinates commonly used in the valence bond order analysis.[17] The 2D proton probability map shows a "double peak" feature that is remarkably similar to those seen for some O···H···O LBHBs.[9,16] For example, Durlak and Latajka[16c] reported very similar results for benzoylacetone. They further showed that the two "peaks" seen in classic ab initio MD simulations merge into one in path-integral MD simulations which take full account of the nuclear quantum effects. Thus, our MD data support the conclusion about the LBHB formation in NA. In Figure 3b, the theoretical curve from the valence bond order model is also shown for comparison. Remarkably, the V-shape ridge seen in the MD proton probability distribution matches the prediction from the valence bond order model. Since Limbach et al.[10b] showed that this theoretical curve is consistent with all known neutron diffraction structures containing OHN HBs, the good agreement found between the valence bond order model and our MD results provides further evidence for the validity of the MD simulations.

Finally we return to the ΔH^{HB} value measured for NA. If we use the three HB energy categories defined by Hibbert and Emsley:[1b] very strong ($> 24\ kcal\,mol^{-1}$), strong (12–24 $kcal\,mol^{-1}$), and weak (2–12 $kcal\,mol^{-1}$), the LBHB enthalpy in NA is clearly not strong. This immediately suggests that the common belief that all LBHBs must be strong is incorrect. It is entirely possible that the LBHB energy can vary over a considerable range. We believe that the LBHB in NA may represent the lower limit of LBHB energy. An intriguing new question to be answered is how spectroscopic and structural characteristics of a LBHB can be quantitatively correlated to its energetics. The results reported in this study will help improve our understanding of the role of LBHB in enzyme catalysis. We are currently using this new ^{17}O NMR approach to investigate the upper limit of LBHB energetics.

Acknowledgements

This work was supported by NSERC of Canada. A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by the National Science Foundation Cooperative Agreement number DMR-1157490, the State of Florida, and the U.S. Department of Energy. We thank Dr. Victor Terskikh (Canadian National Ultrahigh Field NMR Facility for Solids) for acquiring the ^1H MAS spectrum at 21 T and for performing preliminary CASTEP computations.

Conflict of interest

The authors declare no conflict of interest.

Keywords: energetics · low-barrier hydrogen bonds · molecular motion · oxygen · solid-state NMR spectroscopy

How to cite: *Angew. Chem. Int. Ed.* **2017**, *56*, 6166–6170
Angew. Chem. **2017**, *129*, 6262–6266

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Manuscript received: January 15, 2017
Final Article published: February 22, 2017

