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Two-Dimensional NMR Exchange Spectroscopy Study of Proton Exchange in Aqueous Ammonium Ion: H/D Primary Kinetic Isotope Effect for Direct Nitrogen-to-Nitrogen Proton Transfer

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Abstract: Proton exchange between [^{15}N]ammonium ion and ammonia was studied by quantitative two-dimensional NMR exchange spectroscopy (2D-EXSY) in H_2O–D_2O. In this mixture the ammonium ion exists as the five isotopologues $^{15}NH_4^+$, $^{15}NH_3D^+$, $^{15}NH_2D_2^+$, $^{15}NHD_3^+$, and $^{15}ND_4^+$, four of which are distinguishable by ^{1}H NMR with deuterium decoupling. Ammonia-catalyzed exchange is observed even near pH 1, and exchange among all sites is detected as 2D-EXSY cross peaks connecting each pair of species. Exchange with water also occurs and its rate was measured by saturation transfer. Analysis of peak intensities led to the matrix **R**, containing all site-to-site rate constants k_{nm}. Averaging 44 of these gave $(1.6 \pm 0.2) \times 10^8$ M^{-1} s^{-1} as the second-order rate constant per proton for NH_3-catalyzed exchange. The six pairs of rate constants that lie adjacent to the diagonal of **R** contain kinetic information regarding the primary deuterium kinetic isotope effect. From 12 separate estimates a value of $k_H/k_D = 1.8 \pm 0.2$ was calculated for this direct nitrogen-to-nitrogen proton transfer. The low kinetic isotope effect is discussed in terms of a reaction that is nearly encounter-controlled.

Introduction

Some of the simplest and most fundamental proton-transfer reactions are those of aqueous ammonium ion. There are three possible mechanisms (eqs 1–3), depending on the base that

$$NH_4^+ + OH^- \xrightarrow{4k_{OH^-}} NH_3 + H_2O \tag{1}$$

$$NH_4^+ + H_2O \xrightarrow{4k_1} NH_3 + H_3O^+ \tag{2}$$

$$NH_4^+ + NH_3 \xrightarrow{4k_2} NH_3 + NH_4^+ \tag{3}$$

removes the proton.[1] The factor of 4 is introduced because there are four protons per ammonium ion, and it converts all rate constants, second-order, first-order, and second-order, respectively, to a per-proton basis. The hydroxide-catalyzed reaction (eq 1) is thermodynamically very favorable and therefore encounter-controlled.[2] However, its rate can be made negligible by reducing the pH. The water reaction (eq 2) is thermodynamically unfavorable, so that its reverse is encounter-controlled. Thus the forward reaction is limited to a low rate that can be measured independently so that its contribution can be subtracted. Only the thermoneutral transfer (eq 3) is kinetically interesting, since its rate is not determined merely by thermodynamics and encounter control. In contrast to proton transfer between two trimethylamines, which proceeds via a water bridge,[1] this is a direct nitrogen-to-nitrogen proton transfer. By working at pH near 1, so as to adjust the concentrations of OH^- and NH_3, the contribution of this mechanism can be made dominant and brought into a range measurable by nuclear magnetic resonance (NMR) methods.

Kinetic isotope effects (KIEs)[3] are a sensitive probe of transition-state structure. The secondary KIE, k_1^H/k_1^D, for the water-catalyzed exchange (eq 2) was found to be 1.07 ± 0.01.[4] For the direct transfer (eq 3), what is the primary KIE, k_2^H/k_2^D, associated with replacing the hydrogen that is transferred with deuterium? This is a reaction whose barrier may be only 1.5 kcal/mol, although the value calculated is sensitive to basis set and correlation.[5] The reaction may even involve tunneling of the proton across a hydrogen bond between the two nitrogens, so that the KIE might be appreciable. On the other hand there might be none, since the reaction is nearly encounter-controlled. We now report that the primary KIE for this proton transfer is 1.8 ± 0.2.

2D-EXSY Method. In an H_2O–D_2O mixture the ammonium ion exists as the five isotopologues (species differing in isotopic content[6]) NH_4^+, NH_3D^+, $NH_2D_2^+$, NHD_3^+, and ND_4^+, four of which appear in the ^{1}H NMR spectrum. Owing to isotope shifts,[7] separate signals can be distinguished from each of them. To determine the KIE it is necessary to measure and compare site-to-site rate constants for proton transfer from each of those four to any other of them.

It is essential to choose conditions to optimize the determination of the rate constants. A high concentration of ammonium ion is necessary, since NMR is insensitive. The H/D ratio must be chosen so that the NMR signals of all four of the isotopologues are of roughly equal intensity. If either NH_4^+ or NHD_3^+ were weak, too many rate constants would be uncertain. It is imperative

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to use ^{15}N-labeled ammonium ion, since the common ^{14}N undergoes rapid quadrupolar relaxation even in this tetrahedral environment.[8] The ^{15}N has the additional feature of two spin states, each with its own set of ^{1}H signals, so that it is also possible to measure the rate of proton transfer from a nitrogen of one spin state to a nitrogen of the other.

Only quantitative two-dimensional NMR exchange spectroscopy (2D-EXSY) methods[9] are capable of determining all site-to-site rate constants simultaneously. The 2D-EXSY pulse sequence is given in eq 4. The resulting signal depends on both

$$90°–t_1–90°–t_m–90°–t_2(\text{acquire}) \qquad (4)$$

the labeling time t_1 and the acquisition time t_2, as well as on the mixing time t_m. Fourier transformation with respect to both t_1 and t_2 gives a two-dimensional spectrum with a (partially relaxed) one-dimensional spectrum along the diagonal and cross peaks that correspond to site-to-site proton exchange between $NH_nD_{4-n}^+$ and $NH_mD_{4-m}^+$. The intensity of the kl-th peak (diagonal or cross) is given by eq 5,[10] where the ij-th off-diagonal element of

$$I_{kl}(t_m) = (e^{-\mathbf{R}t_m})_{kl} M_l^0 \qquad (5)$$

$-\mathbf{R}$ is k_{ji}, the pseudo-first-order rate constant for exchange from site j to site i, and the diagonal element $R_{ii} = T_{1i}^{-1} + \Sigma_j R_{ij}$, with T_{1i} the spin–lattice relaxation time of nuclei in site i, and where M_l^0 is the equilibrium z-magnetization of nuclei in the l-th site. Fitting the intensities to the exponential of a matrix is a formidable task, which is simplified by linearizing eq 5 to eq 6,[11] where the

$$\mathbf{R} = -\frac{1}{t_m}\ln \mathbf{A} = -\frac{1}{t_m}\mathbf{X}\ln \Lambda\, \mathbf{X}^{-1} \qquad (6)$$

elements of $\mathbf{A}$ are given by $A_{kl} = I_{kl}(t_m)/M_l^0$, $\mathbf{X}$ is the square matrix of eigenvectors that diagonalizes $\mathbf{A}$ to Λ (i.e., $\mathbf{X}^{-1}\mathbf{A}\mathbf{X} = \Lambda$), and $\ln \Lambda = \text{diag}(\ln \lambda_i)$, containing the logarithms of eigenvalues of $\mathbf{A}$. Thus it is possible to obtain all the site-to-site rate constants as elements of $\mathbf{R}$.

In principle the OH peak could be included as a ninth site, but under our conditions, with added Gd^{3+}, it relaxes too rapidly to show cross peaks. Consequently it is more convenient to evaluate the rate constant for exchange with water by saturation transfer.[12] The average first-order rate constant for exchange from NH to OH is given by eq 7,[13] where $I_{NH}\{OH\}$ is the sum of the intensities

$$k_{NH\to OH} = \frac{I_{NH}^{\ 0} - I_{NH}\{OH\}}{I_{NH}^{\ 0}T_{1NH}\{OH\}} \qquad (7)$$

of all the NH peaks on saturating OH, I_{NH}^0 is the equilibrium sum, and $T_{1NH}\{OH\}$ is the apparent spin–lattice relaxation time of NH while saturating OH.

Since the kinetic method is ^{1}H NMR, which leaves deuteron transfer invisible, it would seem as though a KIE is indeterminate. However, the rate constants for exchange between adjacent isotopologues $NH_nD_{4-n}^+$ and $NH_{n-1}D_{5-n}^+$ do carry information about the rate of deuteron transfer. Besides the contribution due to the direct proton transfer between these isotopologues there is an additional indirect contribution. This is due to the apparent exchange of those protons that remain on the nitrogen when a deuteron replaces a proton, or vice versa. Those protons do appear to exchange, since they find themselves on the neighboring isotopologue. This contribution is affected by any primary KIE on the transfer. If k_2^H is much larger than k_2^D, that contribution will be undetectable. Therefore the KIE can be evaluated from the 2D spectrum. Some preliminary results, based on 360-MHz NMR data obtained under slightly different conditions, have been published.[14]

Experimental Section

Sample Preparation. To prepare a 3.20 M $^{15}NH_4NO_3$ solution in 0.071 M HCl at a mole fraction $X_D = 0.431$, 0.400 g of [^{15}N]ammonium nitrate (Isotec), 0.051 g of LiCl, and 0.10 mL of 0.01 M aqueous $Gd(NO_3)_3$ were added to a mixture of 0.40 mL of H_2O, 0.60 mL of 99.8% D_2O, and 0.11 mL of 1.00 N HCl. The presence of 0.78 M LiCl served for spectrometer lock to ensure long-term stability against field drift, and the 6.5×10^{-4} M Gd^{3+} decreased spin–lattice relaxation times so that the pulse sequence could be repeated without an inordinate delay time.

From the stoichiometric H/D ratio, the statistical weights (binomial coefficients), and the fractionation factor $\phi_{NH_4^+} = [ND^+][OH]/[NH^+][OD]$ of 1.107[15] the concentrations $[NH_4^+]$, $[NH_3D^+]$, $[NH_2D_2^+]$, $[NHD_3^+]$, and $[ND_4^+]$ can be calculated as 0.28, 0.924, 1.14, 0.63, and 0.13 M, respectively. The fractionation of deuterium into ammonium ion reduces X_D in the solvent very slightly, to 0.427. Also, the pK_a of NH_4^+ at this concentration in aqueous (not deuterated) LiCl was found to be 9.62 by measuring the pH of a solution also 2.09 M in NH_3. Correction[16] with fractionation factors, including $\phi_{NH_3} = 1.04$,[15] then implies a pK_a of 9.86 in this H/D mixture, a total NL_3 concentration (L = H or D) of 6.22×10^{-9} M, and individual NH_3, NH_2D, NHD_2, and ND_3 concentrations of 1.11×10^{-9}, 2.59×10^{-9}, 2.00×10^{-9}, and 0.52×10^{-9} M, respectively. These concentrations are needed to convert the observed pseudo-first-order rate constants into second-order rate constants for proton transfer from ammonium ion to ammonia. Notice that $[NL_3]$ is quite low, but it is sufficient to catalyze proton exchange at a measurable rate.

NMR Spectroscopy. All ^{1}H spectra were obtained on a Varian UN500 spectrometer operating at 499.843 MHz. The temperature was 25.0 °C, measured by a thermocouple within the probe. No solvent suppression was used. Field-frequency lock to ^{7}Li was effected by the following modifications: The X-band coil of the inverse-detection probe was tuned to the ^{7}Li frequency of 194.26 MHz. The 61.735-MHz output from the lock transmitter was mixed with 255.995 MHz from the frequency synthesizer normally used for the decoupler, filtered, and applied to the X-band coil of the probe. The lock output from that coil was filtered, amplified, and then mixed with the same 255.995 MHz. This output was filtered and applied to the input of the lock preamp. This lock permitted a deuterium-decoupling frequency to be applied through the deuterium-lock coil.

The 2D-EXSY spectrum was obtained using the instrument's NOESY pulse sequence, with hypercomplex phase cycling to produce pure absorption-mode spectra in both dimensions.[17] This is necessary for quantitative intensities to be reliable. Acquisition parameters were a 65.8-μs 90° pulse (unusually long owing to the high dielectric constant and ionic strength of the medium), 1765 t_1 values incremented to a maximum of 1.00 s, a mixing time t_m of 0.5 s, 2048-point FIDs along t_2 (zero-filled to 4096), a sweep width of 1764.3 Hz, 8 acquisitions per phase cycle, and a 3.5-s delay between pulse sequences to allow for complete relaxation before repetition. The large number of t_1 values was needed for adequate resolution in that dimension, and the experiment required a total time of 80 h. The phase settings were selected by phasing the t_1 = 0 spectrum. Peak intensities were evaluated by integrating peak volumes over both dimensions. Peak heights are less sensitive to phasing and to peak overlap, so these were also evaluated. For $T_1\{OH\}$ measurements a sweep width of 2823 Hz, a 10-s relaxation delay, and a line broadening of 0.3 Hz were used.

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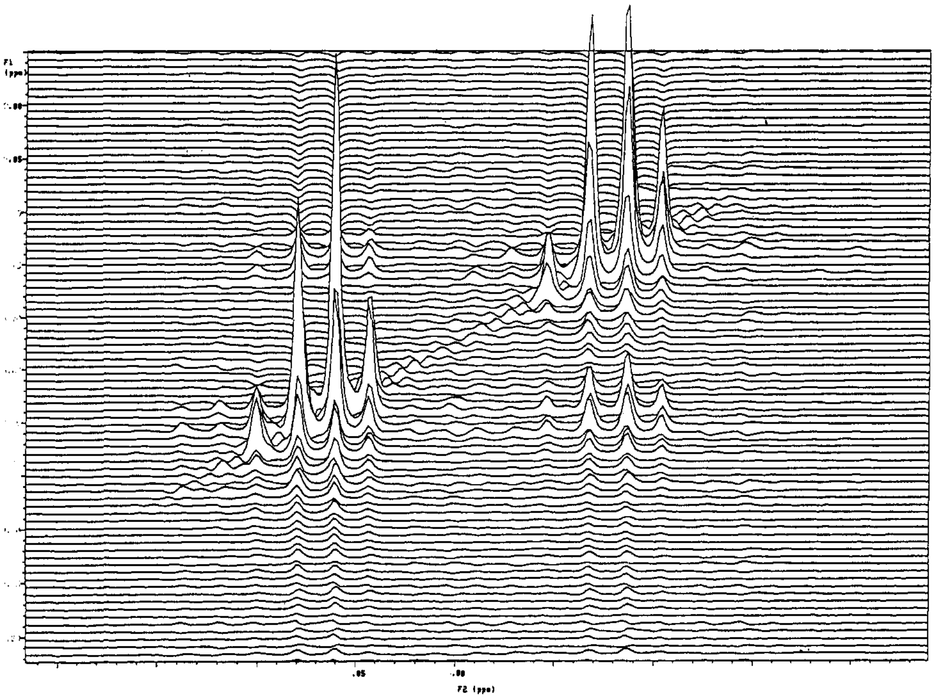

Figure 1. NH region of the pure absorption-mode 2D-EXSY spectrum of ammonium ion isotopologues. The cross peaks correspond to proton exchange between $NH_nD_{4-n}^+$ and $NH_mD_{4-m}^+$.

Data Analysis. In principle equilibrium magnetizations M_l^0 could be determined by repeating the 2D experiment with $t_m = 0$, but it was much less demanding to evaluate their relative magnitudes from the theoretical intensities, $n[NH_nD_{4-n}^+]$, based on stoichiometry. Values for NHD_3^+, $NH_2D_2^+$, NH_3D^+, and NH_4^+ were 0.629, 2.287, 2.7725, and 1.120. For calculations involving peak heights, the intensities in a 1D spectrum were used, but these are less reliable since they do not necessarily have the same broadening as in the 2D spectrum.

Instead of diagonalizing the asymmetric $\mathbf{A} = \mathbf{I}\mathbf{M}_0^{-1}$, where $(\mathbf{I})_{kl} = I_{kl}(t_m)$ and $\mathbf{M}_0^{-1} = \mathrm{diag}(1/M_l^0)$, it is more convenient to diagonalize $\mathbf{M}_0^{-1/2}\mathbf{I}\mathbf{M}_0^{-1/2}$. Then eq 6 becomes eq 8,[18a] where $\mathbf{M}_0^{-1/2}\mathbf{X}$ is the matrix

$$\mathbf{R} = -\frac{1}{t_m}\mathbf{M}_0^{\ 1/2}\mathbf{M}_0^{\ -1/2}\mathbf{X}\ \ln\Lambda\ (\mathbf{M}_0^{\ -1/2}\mathbf{X})^{-1}\mathbf{M}_0^{\ -1/2} \qquad (8)$$

that diagonalizes $\mathbf{M}_0^{-1/2}\mathbf{I}\mathbf{M}_0^{-1/2}$ to Λ. Although $\mathbf{I}$ and $\mathbf{M}_0^{-1/2}\mathbf{I}\mathbf{M}_0^{-1/2}$ are theoretically symmetric, experimental errors make them asymmetric, so they were symmetrized by averaging their off-diagonal elements before the diagonalization. Corresponding pairs of diagonal intensities were also averaged. These symmetrizations avoid the unrealistic complication of complex eigenvectors, but at the expense of not being able to verify independently that forward and reverse rates are equal. A Fortran program was written to carry out these calculations on the 8 × 8 matrices.

Results

Figure 1 shows the NH region of the pure absorption-mode 2D spectrum. The eight-line pattern in each dimension arises because the ^{15}N–H coupling constant of −73.6 Hz splits the signal into a wide doublet, split further by the isotope shift of −20 ppb. This is known to be an unusual case where the deuterium-induced shift is downfield,[19] so that peaks from left to right are NHD_3^+, $NH_2D_2^+$, NH_3D^+, and NH_4^+, first with ^{15}N nuclear spin quantum number $I_z = +^1/_2$ and then with $I_z = -^1/_2$.

The peaks are well enough separated that the intensity of each can be evaluated, although some overlaps, especially of stronger peaks with weaker ones, may create systematic error. Table 1 lists intensities of all NH peaks.

Table 1. Intensities (arbitrary units) of (symmetrized) 2D EXSY Peaks between $NH_nD_{4-n}^+$ and $NH_mD_{4-m}^+$

	m =							
n	1^a	2^a	3^a	4^a	1^b	2^b	3^b	4^b
1^a	0.4215	0.3825	0.2165	0.075	0.022	0.0765	0.0815	0.0325
2^a	0.3825	1.971	1.2295	0.268	0.0685	0.246	0.266	0.138
3^a	0.2165	1.2295	2.424	0.802	0.0765	0.299	0.338	0.148
4^a	0.075	0.268	0.802	0.8315	0.0395	0.124	0.142	0.075
1^b	0.022	0.0685	0.0765	0.0395	0.4215	0.370	0.2015	0.0845
2^b	0.0765	0.246	0.299	0.124	0.370	1.971	1.1415	0.2535
3^b	0.0815	0.266	0.338	0.142	0.2015	1.1415	2.424	0.6885
4^b	0.0325	0.138	0.148	0.075	0.0845	0.2535	0.6885	0.8315

[a] $I_z(^{15}N) = +^1/_2$. [b] $I_z(^{15}N) = -^1/_2$.

The site-to-site rate constants from the relaxation matrix **R** are displayed in Table 2. The diagonal elements of **R** are omitted, since the ignorance of absolute intensities has multiplied them by an arbitrary constant, thereby losing the relaxation information while leaving the rate constants invariant.[11] Owing to inaccuracies in integration due to peak overlap and propagation of errors through the matrix algebra, the values in Table 2 are rather inaccurate.

Saturation of the OH resonance produces a diminution $(I_{NH}^0 - I_{NH}\{OH\})/I_{NH}^0$ of 17.6% in the total height of all the NH peaks, whose average apparent spin–lattice relaxation time $T_{1NH}\{OH\}$, evaluated by weighted linear least squares, is 0.572 s. Equation 7 then leads to an average rate constant of 0.31 s^{-1} for exchange of NH protons into water. This is considerably lower than the value of 2.5 s^{-1} previously observed at 3.0 M salt, but the rate constant does decrease with ionic strength.[1] From the populations and the known[4] secondary KIE on k_1, it is then possible to convert this average into the rate constants for exchange of NH protons between each $NH_nD_{4-n}^+$ and H_2O. These are also included in Table 2.

Discussion

Relations between Observed (Macroscopic) and Mechanistic (Microscopic) Rate Constants. Equation 9, where δ_{nm} is 1 if *m*

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Table 2. Rate Constants (s^{-1}) for Proton Exchange from $NH_nD_{4-n}^+$ or OH to $NH_mD_{4-m}^+$ or OH

	$m =$								
n	1^a	2^a	3^a	4^a	1^b	2^b	3^b	4^b	OH
1^a		1.628	0.31	0.196	0.047	0.161	0.168	0.041	0.34
2^a	0.448		1.398	0.02	0.037	0.135	0.116	0.095	0.315
3^a	0.07	1.154		0.82	0.024	0.138	0.158	0.052	0.295
4^a	0.11	0.041	2.028		0.053	0.125	0.132	0.108	0.275
1^b	0.047	0.133	0.106	0.095		1.562	0.295	0.257	0.34
2^b	0.044	0.135	0.168	0.061	0.429		1.239	0.063	0.315
3^b	0.038	0.095	0.158	0.053	0.067	1.022		0.657	0.295
4^b	0.023	0.194	0.128	0.108	0.144	0.128	1.626		0.275
OH	0.0038	0.0089	0.0070	0.0018	0.0038	0.0089	0.0070	0.0018	

[a] $I_z(^{15}N) = +^1/_2$. [b] $I_z(^{15}N) = -^1/_2$.

$$k_{nm} = {}^1/_2 k_2^H[NH_{m-1}D_{4-m}] + {}^1/_4 n\delta_{nm}/T_{1^{15}N} \qquad (9)$$

$= n$ and 0 if $m \neq n$, expresses the site-to-site rate constants for proton exchange from $NH_nD_{4-n}^+$ to $NH_mD_{4-m}^+$ for the simple cases where $|n-m| > 1$ or where the ^{15}N spin I_z changes. Equations 10 and 11 account for the special cases of exchange where $|n -$

$$k_{nm-1} = {}^1/_2 k_2^H[NH_{n-2}D_{5-n}] + (n-1)k_1^H P_1^D + (n-1)k_2^H P_2^D[NL_3] \qquad (10)$$

$$k_{nm+1} = {}^1/_2 k_2^H[NH_nD_{3-n}] + (4-n)k_1^D P_1^H + (4-n)k_2^D P_2^H[NL_3] \qquad (11)$$

$m| = 1$ and with $\Delta I_z = 0$. The mechanistic rate constants are those of eqs 2 and 3, but with superscripts to designate whether a proton or a deuteron is transferred. Also $P_i^D = 1 - P_i^H = k_i^D X_D/(k_i^H X_H + k_i^D X_D) = (X_D/X_H)/(k_i^H/k_i^D + X_D/X_H)$, which represents the probability in each of the two pathways ($i = 1, 2$) that a deuteron will be transferred to the nitrogen.

To derive these relationships between the observed site-to-site rate constants and the mechanistic rate constants requires proper attention to probabilities. The first term in eq 9 represents proton transfer from $NH_nD_{4-n}^+$ to $NH_{m-1}D_{4-m}$, which thereby becomes $NH_mD_{4-m}^+$. The factor of $^1/_2$ corrects for the probability of each ^{15}N spin state. The second term in eq 9 corrects for spin–lattice relaxation of the ^{15}N, which interconverts the two spin states. This process appears to transfer protons between one ^{15}N with $I_z = +^1/_2$ and another with $I_z = -^1/_2$ even though there are not two different nuclei and therefore no proton transfer. The factor of $^1/_4 n$ corrects for the number of protons on $NH_nD_{4-n}^+$, since deuterium is much less effective at relaxing ^{15}N. The spin–lattice relaxation time of aqueous $^{15}NH_4^+$, $T_{1^{15}N}$, has been measured[20] as 44 s.

When $|n - m| = 1$ there are additional indirect mechanisms for proton exchange that contribute whenever a proton is exchanged for a deuteron. The first terms of eqs 10 and 11 represent the contribution of the direct proton transfer, as in eq 9. The middle term of eq 10 represents the contribution from proton transfer to water, followed by deuteronation, and the middle term of eq 11 represents the contribution from removal of one of the $4 - n$ deuterons by water, followed by protonation. These contributions do not come from the proton (or deuteron) that is transferred but from those protons that remain on the nitrogen (a total of $n - 1$). Likewise, the third terms of eqs 10 and 11 represent the contribution from proton removal by NL_3 followed by deuteronation, or vice versa. These processes also transfer to an adjacent isotopologue the $n - 1$ protons that remain on the nitrogen, since the number of protons increases or decreases by one.

Equation 12 expresses the requirement that forward and reverse rates must be identical for all n and m. This equation is satisfied, term-by-term, by the rate constants in eqs 9–11. To verify this,

$$nk_{nm}[NH_nD_{4-n}^+] = mk_{mn}[NH_mD_{4-m}^+] \qquad (12)$$

it is necessary to remember that the statistical weights of $NH_nD_{4-n}^+$ and NH_nD_{3-n} are $4!/n!(4-n)!$ and $3!/n!(3-n)!$, respectively.

Table 3. Primary KIE for Proton Exchange, from Analysis of Eqs 10 and 11 (Neglecting Fractionation Factors and Secondary KIEs)

n	m	k_{nm}, s^{-1}	term 1, s^{-1}	term 2, s^{-1}	term 3, s^{-1}	P_2^D	k_2^H/k_2^D
2	1	0.448[a]	0.043	0.131	0.274	0.268	2.037
1	2	1.628[a]	0.165	0.529	0.934	0.227	2.541
3	2	1.154[a]	0.165	0.263	0.726	0.355	1.354
2	3	1.398[a]	0.213	0.353	0.833	0.303	1.713
4	3	2.028[a]	0.213	0.394	1.421	0.463	0.864
3	4	0.820[a]	0.091	0.176	0.552	0.402	1.108
2	1	0.429[b]	0.043	0.131	0.255	0.249	2.245
1	2	1.562[b]	0.165	0.529	0.868	0.211	2.790
3	2	1.022[b]	0.165	0.263	0.594	0.290	1.821
2	3	1.239[b]	0.213	0.353	0.674	0.245	2.293
4	3	1.626[b]	0.213	0.394	1.019	0.332	1.499
3	4	0.657[b]	0.091	0.176	0.389	0.283	1.884

The second-order rate constant k_2^H for NH_3-catalyzed proton exchange was determined using eq 9, the individual $NH_{m-1}D_{4-m}$ concentrations, the small correction for spin–lattice relaxation of the ^{15}N, and the 44 rate constants k_{nm} in Table 2 for which $|n - m| > 1$. A value of $(1.6 \pm 0.2) \times 10^8$ M^{-1} s^{-1} per proton was obtained. Although this value is based on the inaccurate data in Table 1, the average of 22 pairs of such values is less inaccurate, and it is the standard error of the mean that is reported. However, this rate constant is slightly lower than the 3.0×10^8 M^{-1} s^{-1} per proton observed by Emerson, Grunwald, and Kromhout.[1]

Kinetic Isotope Effect. According to eqs 10 and 11, the six pairs of site-to-site rate constants k_{nm} for which $|n - m| = 1$ and $\Delta I_z = 0$ contain kinetic information from both NH_3- and H_2O-catalyzed proton removal, and these are subject to a primary deuterium KIE. These rate constants are repeated in Table 3. The contributions of the first term in each equation can be evaluated using the above k_2^H and the individual $NH_{n-2}D_{5-n}$ and NH_nD_{3-n} concentrations. The contributions of the second terms can be evaluated using the average k_1^H of 0.31 s^{-1}. The primary KIE, k_1^H/k_1^D, is taken as 1 since according to the Swain–Grunwald mechanism[21] the rate-limiting step under weakly acidic conditions is the diffusional separation of a hydron, and to a first approximation this rate does not depend on whether there is a hydrogen or a deuterium in the hydrogen bond between water and ammonia. These two terms are also listed in Table 3.

Both of these contributions represent small corrections, especially the first one. Subtracting them from the observed k_{nn-1} or k_{nn+1} provides the third terms, which are listed in Table 3. Then from the known $[NL_3]$ and the k_2^H evaluated above, P_2^D and $k_2^D P_2^H = (X_H/X_D)k_2^H P_2^D$ can be evaluated, and from that the primary KIE, $k_2^H/k_2^D = (X_D/X_H)(1/P_2^D - 1)$. The computations were facilitated by the use of an Excel spreadsheet

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Table 4. Primary KIE for Proton Exchange, from Analysis of Eqs 10 and 11

n	m	k_{nm}, s^{-1}	term 1,[c] s^{-1}	term 2,[d] s^{-1}	term 3,[e] s^{-1}	P_2^D	k_2^H/k_2^D
2	1	0.448[a]	0.044	0.111	0.293	0.293	2.053
1	2	1.628[a]	0.159	0.441	1.028	0.292	2.066
3	2	1.154[a]	0.170	0.238	0.746	0.362	1.501
2	3	1.398[a]	0.206	0.315	0.878	0.362	1.498
4	3	2.028[a]	0.219	0.382	1.427	0.447	1.053
3	4	0.820[a]	0.088	0.168	0.563	0.451	1.037
2	1	0.429[b]	0.044	0.111	0.274	0.274	2.255
1	2	1.562[b]	0.159	0.441	0.962	0.273	2.266
3	2	1.022[b]	0.170	0.238	0.614	0.298	2.007
2	3	1.239[b]	0.206	0.315	0.719	0.297	2.018
4	3	1.626[b]	0.219	0.382	1.025	0.321	1.800
3	4	0.657[b]	0.088	0.168	0.400	0.320	1.807

[a] $I_z(^{15}N) = +^1/_2$. [b] $I_z(^{15}N) = -^1/_2$. [c] $^1/_2 k_2^H(\phi_{NH_4^+}/\phi_{NH_3})^{(n-m)/2}[NH_{m-1}D_{4-m}]$. [d] $(n-1)k_{1n}{}^H P_1^D$ or $(4-n)k_{1n}{}^D P_1^H$. [e] $(n-1)k_2^H(\phi_{NH_4^+}/\phi_{NH_3})^{(n-\tilde{m}-1)/2}P_2^D[NL_3]$ or $(4-n)k_2^D(\phi_{NH_4^+}/\phi_{NH_3})^{(n-\tilde{m})/2}P_2^H[NL_3]$.

program, and the stages are compiled as Table 3. From the twelve separate estimates of this KIE in the last column of Table 3 a value of $k_2^H/k_2^D = 1.8 \pm 0.6$ (or ±0.3 = standard error of the mean) was calculated for the reaction of eq 3.

Inclusion of Fractionation Factors and Secondary KIEs. Equations 9–11 ignore secondary KIEs. The data are too inaccurate to permit evaluation of these, so they must be estimated independently from fractionation factors.[16] Then each of the rate constants in eqs 9–11 must be corrected, although all of these corrections are small.

Besides statistical factors, the equilibrium constant for proton transfer from $NH_nD_{4-n}^+$ to $NH_{m-1}D_{4-m}$ includes the factor $(\phi_{NH_4^+}/\phi_{NH_3})^{n-m}$. Since this is a symmetric proton transfer, the transition state must be midway between reactant and product, so that the secondary KIE is the square root of the equilibrium isotope effect. Therefore k_2^H for proton transfer from $NH_nD_{4-n}^+$ to $NH_{m-1}D_{4-m}$ is $(\phi_{NH_4^+}/\phi_{NH_3})^{(n-m)/2}$ times k_2^H for proton transfer specifically from NH_4^+ to NH_3. This substitution[15] must be made in the first term of eq 9. Then from the individual $NH_{m-1}D_{4-m}$ concentrations and the 44 corrected rate constants k_{nm} with $|n-m| > 1$ or with $\Delta I_z = \pm 1$, corrected also for the spin–lattice relaxation time of the ^{15}N, a value of $(1.6 \pm 0.2) \times 10^8$ M^{-1} s^{-1} per proton was obtained. This is the same value as obtained by the simpler calculation above.

The KIE is calculated from the rate constants k_{nm} for which $|n-m| = 1$ and $\Delta I_z = 0$ (eqs 10 and 11), corrected for secondary KIEs. The previous substitution must also be made in k_2^H of the first terms. For the second terms the secondary KIE on k_1^H was measured independently,[4] and the corrected rate constants have been included in Table 2. The primary KIE is not 1 as above but is equal to the equilibrium constant, given by $\phi_{NH_4^+}/\phi_{H_3N\text{-}HOH}$. The denominator is unknown but is taken as 0.95, the average of 0.90 for $\phi_{ArNMe_2H^+\text{-}NMe_2Ar}$[22] and 1 for $\phi_{H_2O\text{-}HOH}$. (Except for strong intramolecular hydrogen bonds,[23] fractionation factors for hydrogen bonds are close to 1,[24] so this estimate cannot be in great error.) Also, in calculating P_1^D and P_1^H it is necessary to take account of $\phi_{H_3N\text{-}HOH}$, which enriches the hydrogen bond in protium, so that X_D/X_H is less than in bulk solvent. Both these terms can be subtracted from the observed rate constants to obtain the third term in eq 10 or 11. Those rate constants and each of the terms are listed in Table 4.

For the third terms of eqs 10 and 11 the secondary KIE on k_2 is the same as that for the first terms, applied above. In particular, the rate constant for proton transfer or deuteron transfer from $NH_nD_{4-n}^+$ to NL_3 is, respectively, $(\phi_{NH_4^+}/\phi_{NH_3})^{(n-\tilde{m}-1)/2}$ times k_2^H for proton transfer specifically from NH_4^+ to NH_3 or $(\phi_{NH_4^+}/\phi_{NH_3})^{(n-\tilde{m})/2}$ times k_2^D for deuteron transfer specifically from NH_3D^+ to NH_3, where $\tilde{m}$ is 1.69, the average deuterium content of NL_3 in the sample. Also P_2^D depends on the isotopic ratio in the ammonium ion, which differs from the ratio X_D/X_H in the solvent by the fractionation factor $\phi_{NH_4^+}$. Then from $[NL_3]$, the known fractionation factors, and the k_2^H evaluated above, P_2^D and $k_2^D P_2^H = (X_H/X_D)k_2^H P_2^D$ can be evaluated, and from that the ratio k_2^H/k_2^D. Actually this ratio applies to L_2NHD^+, which is subject to a secondary KIE, so it is necessary to multiply it by $(\phi_{NH_4^+}/\phi_{NH_3})^{1/2}$. This gives the desired primary KIE as the ratio of k_2^H for proton transfer from NH_4^+ to NH_3 to k_2^D for deuteron transfer from NH_3D^+ to NH_3. The computations were facilitated by the use of an Excel spreadsheet program, and the stages are compiled as Table 4. It is clear that the corrections for secondary KIEs and fractionation factors are all small.

From the twelve separate estimates of this KIE in the last column of Table 4 an average value of $k_2^H/k_2^D = 1.8$ was obtained for the reaction of eq 3. Although this is based on inaccurate data, leading to a standard deviation of 0.4, the average of six pairs of values is less inaccurate, and the standard error of the mean is only 0.2. The same k_2^H/k_2^D, with a slightly larger error of 0.3, was obtained using peak heights instead of integrals.

There are systematic errors in the values. In Table 3 the k_2^H/k_2^D calculated from rate constants k_{nm} with $n > m$ are less than those with $n < m$. However, this error disappears in Table 4, when fractionation factors and secondary KIEs are taken into account. The remarkably good agreement is good evidence that these corrections have been applied consistently. A more serious error in both tables is the systematic decrease of k_2^H/k_2^D with increasing n. This cannot be due to systematic errors in the corrections for the direct exchange or the water-catalyzed reaction, since these corrections are too small. It might be due to errors in measuring the peak intensities adjacent to the diagonal of the 2D spectrum. However, it does not affect the primary KIE but only increases its error. Another source of error lies in the covariance of the separate estimates of the KIE. An exact error analysis is too formidable a task, since errors propagate through the matrix algebra of eq 8. As a result the estimated error of ±0.2 in k_2^H/k_2^D may be an underestimate. However, even if the error is considerably larger, it does not appreciably change the conclusion that k_2^H/k_2^D is near 2.

A Small Primary KIE. The KIE is definitely small. This can be seen from comparing the observed rate constants in column 3 of Table 3 or 4 with the sum of rate constants in columns 4 and 5. This sum is the rate constant expected from the direct proton transfer plus the water-catalyzed exchange (eq 2). If the KIE were infinite, there would be no additional contribution to the observed rate constants. Since that additional contribution is quite large compared to that sum, the KIE must be rather small. Alternatively, this conclusion follows from the spectrum in Figure 1. The twelve 2D-EXSY cross peak intensities adjacent to the diagonal are diagnostic for an appreciable contribution from an additional pathway, besides direct proton transfer, for exchange between adjacent isotopologues $NH_nD_{4-n}^+$ and $NH_{n-1}D_{5-n}^+$. They are considerably greater than the other cross peak intensities. For the KIE to be as high as 5.0, inversion of the calculations shows that the average of those twelve intensities would need to be only 60% of what is observed. So large an error in evaluating cross peak intensities is out of the question.

It is unlikely that the KIE for eq 3 is low because of other sources of error. The value calculated is quite sensitive to the correction for water-catalyzed exchange (eq 2). This is measured independently by saturation transfer, and its rate constant is unlikely to be in error. The correction also depends on the magnitude of the primary KIE for eq 2, and this relies on the Swain–Grunwald mechanism, whose interpretation has been

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questioned.[25] However, if this primary KIE is actually large, as for other proton transfers, then the KIE for eq 3 would be smaller than what we have calculated.

Encounter Control? A value of 1.8 is quite low for a primary KIE. However, since $4k_2$ is 6.6×10^8 M^{-1} s^{-1}, this reaction is nearly encounter-controlled. So fast a reaction is consistent with a low barrier to hydron transfer.[5] To the extent that the rate-limiting step is the encounter of the ammonium ion with the ammonia, the hydron transfer is not rate-limiting. Then the rate of encounter would be independent of isotope, and the KIE would be unity. If k_L in eq 13[2]

$$H_3NL^+ + NH_3 \underset{k_{sep}}{\overset{k_{enc}}{\rightleftarrows}} \overline{H_3NL^+ + NH_3} \xrightarrow{k_L} \overline{NH_3 + LNH_3^+} \quad (13)$$

is high compared to k_{sep}, the rate constant for separation of the encounter pair, then the observed rate constant will be simply k_{enc}, regardless of whether L = H or D.

Such behavior has been seen[26] in hydron transfer from catalyzing acid to $CH_3ONH_2^+$–CH(OPh)–O$^-$ or $CH_3ONH_2^+$–CHAr–O$^-$. Only if the acid is of matching pK_a is the KIE large, since otherwise diffusion becomes rate-limiting. However, that maximum observed KIE is 3–4, and the KIE estimated for the actual hydron transfer may be as high as 12, significantly larger than our 1.8. This discrepancy is serious, since this direct oxygen-to-oxygen proton transfer ought to be most comparable to our nitrogen-to-nitrogen transfer.

Besides, there are further quantitative difficulties. Even a low estimate[27] for k_{enc} of 1.7×10^9 M^{-1} s^{-1} is considerably higher than the observed $4k_2$. This lattter is $k_{enc}k_H/(k_{sep} + k_H)$ in eq 13, so that the ratio k_H/k_{sep} cannot be larger than 0.6. The observed KIE then corresponds to a k_H/k_D for the actual proton transfer of only 2.3, which is still unusually small. However, this estimate is quite sensitive to the values of k_{enc} and of [NL_3], which depends on the pK_a of NH_4^+ in the NMR sample.

A truly encounter-controlled reaction would show an interesting feature. For such a reaction k_{sep} in eq 13 would be small compared to k_L, so that whenever the encounter complex is formed, it always leads to hydron transfer. Yet even after the transfer, the encounter complex is composed of an ammonium ion and an ammonia, capable of further transfers, as in eq 14. If the reaction were

$$L_3NH^+ + NL_3 \rightarrow \overline{L_3NH^+ + NL_3} \rightarrow$$
$$\overline{NL_3 + HNL_3^+} \rightarrow \overline{L_4N^+ + HNL_2} \rightarrow \text{etc.} \quad (14)$$

truly encounter-controlled, all seven hydrons would be scrambled between the two nitrogens before the encounter complex separates. Such scrambling, although only partial, has been seen in the gas-phase reaction of carbanions with D_2O[28] and also in hydron transfer between benzenes.[29]

It is possible to demonstrate independently that the reaction is not fully encounter-controlled. Complete scrambling of hydrons between the two nitrogens, followed by eventual return back to ammonium ion of any protons transferred to ammonia, can be treated as a Markov chain.[18b] The resulting pattern of site-to-site rate constants is rather uniform, without the large $k_{nn\pm1}$ observed. It was not possible to test whether this feature would be manifested in viscous media, where the lifetime of the encounter pair is longer, since in ethylene glycol the NH peaks broaden so much that they overlap and preclude the quantitative 2D measurements.

It is still puzzling why the KIE is substantially lower than in a similar reaction involving oxygen-to-oxygen transfer.[26] The difference may be that in that case the reactants are already hydrogen-bonded, so that the hydron transfer can readily occur. In hydron transfer from ammonium ion to ammonia it may further be necessary to rotate the reactants into position. If so, this rotation would also be partially rate-limiting, and independent of isotope, so that the KIE would be reduced. Rotation of ammonium ion within an aqueous solvent cage is known to be very fast, requiring only 1 ps,[20] but with other solvents it can be slower.[30] It is perhaps less likely that the ammonium ion rotation is retarded in the vicinity of an ammonia molecule, but this latter may be slower to rotate. Another possible reason for the low KIE is the necessity for reorganizing the solvent to accommodate the shift of the positive charge.[31] If this retards the hydron transfer, the rate will be less dependent on isotope. The possible roles of rotation and solvent reorganization in the aqueous ammonium ion–ammonia complex represent a challenge to molecular modeling.

Conclusions and Summary. The KIE is definitely low, near 1.8. In part this is due to the fact that the hydron transfer is not completely the rate-limiting step. Instead, the reaction is nearly encounter-controlled, and there may also be a contribution from the need to reorganize the solvent or rotate the ammonia or ammonium ion so that the hydron can be transferred.

This is the first example of a primary KIE for a direct nitrogen-to-nitrogen proton transfer in solution. Moreover, it is the first primary KIE determined directly in the H_2O–D_2O mixture. What makes this possible is 2D-EXSY spectroscopy, which determines the site-to-site rate constant for proton transfer from each ammonium ion isotopologue to each other.

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