the primed coordinate system. The angles β and β' will depend on θ in some fashion. Using a standard Euler matrix,[35] and allowing the arbitrary angle α to be 0, we have

$$S_x = S_{x'} \cos \beta - S_{z'} \sin \beta$$
$$S_y = S_{y'}$$
$$S_z = S_{x'} \sin \beta + S_{z'} \cos \beta$$

where we have assumed axial symmetry. The angle γ is then arbitrary and we have set it equal to 0°. The expressions for I_x, I_y, and I_z may be obtained by replacing the S's by I's, and β by β'. The spin Hamiltonian then becomes

$$\mathcal{H} = \beta H_0\{(g_{||} \cos\theta \cos\beta - g_\perp \sin\theta \sin\beta)S_{z'} + (g_{||} \cos\theta \sin\beta + g_\perp \sin\theta \cos\beta)S_{x'}\} + (D/2)\Big\{[S_{z'}^2 - {}^1/_3 S(S+1)] \cos^2\beta + \frac{(S_{+'}^2 + S_{-'}^2)}{2} \sin^2\beta + [(S_{+'} + S_{-'})S_{z'} + S_{z'}(S_{+'} + S_{-'})] \sin\beta \cos\beta\Big\} + [A_\perp \sin\beta \sin\beta' + A_{||} \cos\beta \cos\beta')S_{z'}I_{z'} + [A_{||} \sin\beta \sin\beta' + A_\perp(\cos\beta \cos\beta' - 1)]\frac{(S_{+'}I_{+'} + S_{-'}I_{-'})}{4} + [A_{||} \sin\beta \sin\beta' + A_\perp(\cos\beta \cos\beta' + 1)] \times \frac{(S_{+'}I_{-'} + S_{-'}I_{+'})}{4} + (A_{||} \cos\beta' \sin\beta' - A_\perp \sin\beta' \cos\beta')[(S_{+'} + S_{-'})I_{z'}]$$

by making the usual substitution of raising and lowering operators for x and y operators. One additional constraint we have applied is that the coefficient of the term in $S_{z'}I_{x'}$ should be zero, as previously mentioned. This condition leads to a relationship between β and β', namely $A \tan\beta' = B \tan\beta$, but no general expression is available to relate θ to β or β'.

The spin Hamiltonian derived in this way is almost of the same form as that found when $D \ll g\beta H$. One difference is the additional term in $S_{x'}$ in the Zeeman interaction, but this does not affect the form of the perturbed wave functions. We assume the following relationship between θ and β and β'

$$\sin\beta = K_1 \sin\theta \qquad \cos\beta = K_3 \cos\theta$$
$$\sin\beta' = K_2 \sin\theta \qquad \cos\beta' = K_4 \cos\theta$$

When $D \ll g\beta H$, the K_i's are products of $g_{||}$ and $g_\perp$ and A and B, the hyperfine constants. We cannot determine K_i here, as we cannot set any term in $\mathcal{H}$ equal to zero. Thus we have no additional equations relating θ, β, and β'. The field at resonance is a function of sin β and cos β, and since we do not know the relation between β and θ, this precludes using $\mathcal{H}$ to determine H_{res} *vs.* θ by a perturbation calculation. We do assume, though, that at $\theta = 0°$, all K_i's are unity. This allows us to use data at $\theta = 0°$ to obtain some parameters, although we generally use the standard Hamiltonian for $D \ll g\beta H$ in this type of calculation.

(35) M. E. Rose, "Elementary Theory of Angular Momentum," Wiley, New York, N. Y., 1957, p 65.

Magnetic Resonance Measurements of Proton Exchange in Aqueous Urea[1a]

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Abstract: Rates of protolysis of aqueous urea have been measured by steady-state and spin-echo nmr methods, with good agreement between the two techniques. Below about 2 *M* urea, the lifetime of a proton on urea is independent of urea concentration. The rate constants for acid-, base-, and water-catalyzed protolysis are (9.0 ± 1) × 10^6 M^{-1} sec^{-1}, (2.4 ± 1) × 10^6 M^{-1} sec^{-1}, and about 1.5 ± 0.5 sec^{-1}, respectively. Protonation occurs at urea nitrogen rather than oxygen. Above about 2 or 3 *M* urea, formation of aggregated species of urea causes the lifetime of a urea proton to depend on urea concentration.

Magnetic resonance measurements have provided detailed information on the rates and mechanisms of proton transfer in aqueous amines[2a] and amides.[2b] Despite its relevance to theories of solution "structure" and the reversible denaturation of proteins, similar information for urea and substituted ureas is lacking. Most of the nmr rate measurements on amines and amides depend on observation of collapse of spin multiplets of protons coupled to the exchanging protons, and this convenient technique cannot be applied to urea.

In this paper we report measurements of the water and urea nmr line shape. The results are analyzed including explicitly the effect of quadrupole relaxation

(1) (a) We gratefully acknowledge partial support of this work by the Office of Naval Research, under Contract No. N00014-67-A-0200-6021, and the National Science Foundation. (b) Address correspondence to this author.

(2) (a) A. Loewenstein and S. Meiboom, *J. Chem. Phys.*, 27, 1067 (1957); (b) A. Loewenstein and T. M. Conner, *Ber. Bunsenges. Phys. Chem.*, 67, 280 (1963).

of the nitrogen nuclei. Rate constants for acid- and base-catalyzed protolysis of urea are obtained, and the validity of the method is supported by the results of spin-echo nmr measurements.

Experimental Section

Urea (Matheson Coleman and Bell) was twice recrystallized from methanol, keeping the temperature below 50°. Steady-state nmr line shapes were obtained for six urea concentrations between 2 and 10 *M* urea. Below 2 *M*, the line broadening was insufficient for accurate measurement and spin-echo methods were used.

Steady-State Measurements. A Varian T-60 nmr spectrometer operating at 60 MHz and 35 ± 1° was used. The spectra consisted either of a single line or a wide, weak line (urea) and a narrower, more intense (water) line, depending on pH. Because of the wide disparity in integrated intensity and line width, it was impossible to record the complete line shape. Instead, at least four sweeps in alternate directions of urea and water peaks were recorded separately. For lines wider than about 4 Hz, a sweep rate of 1.0 Hz/sec was used, and for narrower lines, a sweep rate of 0.2 Hz/sec. Precalibrated frequency scales were used, the calibration being checked periodically by the side-band method. The rf field was adjusted well below saturation for each sample.

Prior to each set of measurements, the rf phase was adjusted to yield a symmetric Lorentzian line for pure degassed water. Nevertheless, the urea line shape was found in most cases to be quite sensitive to minor rf phase adjustment because of overlap with one wing of the very intense water peak. Such behavior is a severe limitation for studies of exchange between two highly unequally populated sites. Only in neutral solution where the water resonance showed no exchange broadening were we able to obtain accurate urea line widths.

None of the samples used for steady state measurements was buffered; pH was adjusted using small amounts of strong hydrochloric acid or sodium hydroxide. The pH of each sample was measured at 35° immediately before and after line-shape measurements. In most cases the pH had not changed by more than 0.02 unit during the 10 min or so required for line-shape measurements. Only such samples were used for kinetic analysis.

Spin-Echo Measurements. The pulsed spectrometer consists of of an NMR Specialties, Inc. P118A rf unit and P102A pulse programmer,[3] an RHG EVT 6020 transistorized receiver, a Tektronix 564 storage screen oscilloscope, and a home-built single coil probe with a center tap rf input for impedance matching. The coil diameter is 10 mm and thick-walled sample tubes of o.d. 8 mm were used to improve rf field homogeneity. The sample volume was kept entirely within the coil. The recovery time following a 17-μsec 90° pulse for protons at 55.0 MHz is about 16 μsec. A Varian HR-60 12-in. high-resolution magnet equipped with flux stabilizer and homogeneity coils was used. For a sample of pure degassed water at ambient probe temperature of 32 ± 2°, T_1 was found to be equal to T_2 and in agreement with literature values[4] within ±5%.

Relaxation curves, or plots of apparent transverse relaxation rate R_2 *vs.* rf pulse spacing 2τ, were obtained for 1 and 2 *M* urea at many pH values between 5.3 and 9.0. Pulse spacings less than 2 msec could not be used because of duty-cycle limitations of the rf unit. For large pulse spacings, a correction for diffusion effects was applied to measured relaxation rates. Prior to each set of measurements, the static field was adjusted to maximum homogeneity by maximizing the length of a free induction decay of pure water. R_2 of pure water was then measured as a function of pulse spacing from 2 to 256 msec. Typically, no dependence on pulse spacing was found between 2 and 20 msec, and the relaxation rate at 256 msec was 10–15% larger than that at 2 msec. The difference between R_2 of pure water at a given pulse spacing and the short pulse spacing limiting value was subtracted from relaxation rates of aqueous urea at the same pulse spacing. This procedure is not exact, but introduces no significant error because the total correction is a small per cent of the total relaxation rate.

It was not practical to measure the pH of each degassed sample immediately before and after measurement of a relaxation curve, so pH was controlled by buffers. NaH_2PO_4–Na_2HPO_4 was used for acid solutions, and borax with hydrochloric acid or sodium hydroxide was used for basic solutions. Measured spin–lattice relaxation rates of the buffer solutions were the same as that of pure water within experimental error, and doubling buffer concentration (at constant pH) did not alter measured transverse relaxation rates of aqueous urea solutions. A total buffer concentration of about 0.05 *M* was used for all kinetic experiments. The pH did not change during degassing or over a period of several hours.

All echo trains were found to be single exponential decays within experimental error, no doubt because of the large excess of water protons. The relaxation rates were estimated from measurements of the trace on the storage oscilloscope, with rf phase controls and resonance offset[5] adjusted to give the longest possible decay.

Measurement of pH. A Corning Model 10 pH meter equipped either with a Corning No. 476050 combination electrode or No. 476010 reference and No. 476020 glass electrode was used for all the pH measurements. Several electrodes of each type were used, all giving consistent results. The pH was assumed to be $-\log [H^+]$; in the pH range used the activity coefficient of hydrogen ion is nearly unity and the sodium ion error is negligible. No correction for bound hydrogen[6] was used because the mole fraction of protonated species of urea is negligibly small.

Data Analysis and Results

In calculating line shapes and spin echoes we assume that protons in aqueous urea undergo exchange between two unequally populated sites, and are scalar coupled in one site to a relaxing nucleus of spin 1. Protons coupled to the quadrupolar nucleus are allowed to scramble their spin states both by relaxation of the quadrupole and a direct exchange process not involving transfer to the other site. The absorption intensity $I(\omega)$ at ω rad/sec from the first moment of the spectrum is then[7]

$$I[\omega] = Re[p_u\alpha + (\beta + \eta)p_w + 4kp_up_w]/[\alpha(\beta + \eta) + 4k^2p_up_w] \quad (1)$$

where

$$\alpha = -R_2^0 - k_{wu} + i(p_u\delta\omega - \omega) \quad (2)$$

$$\beta = -R_2^0 - k_{uw} - i(p_w\delta\omega + \omega) \quad (3)$$

$$\eta = {}^2/_3\mathcal{A}^2(\beta - 0.6R_1 - k_d)/[(\beta - 0.6R_1 - k_d) \times (\beta - R_1 - k_d) + \mathcal{A}^2/_3] \quad (4)$$

Here p_u and p_w are fractional populations of urea and water protons, respectively, and k is the average exchange rate, the arithmetic mean of the inverse lifetimes k_{uw} and k_{wu} of a proton on urea or water, respectively. By detailed balance

$$k_{uw} = 2kp_w \quad (5)$$

$$k_{wu} = 2kp_u \quad (6)$$

$\delta\omega$ is the chemical shift in rad/sec between water and urea, $\mathcal{A}$ is the proton–nitrogen scalar coupling constant in rad/sec, R_1 is the *nitrogen* spin–lattice relaxation rate, R_2^0 is the *proton* relaxation rate in absence of exchange or quadrupole relaxation, and k_d is the rate of direct transfer of protons between nitrogen atoms (not involving water).

We have not obtained closed-form expressions analogous to eq 1–6 for decay rates in Carr–Purcell spin-echo[8] experiments. Instead, relaxation curves were calculated by numerical diagonalization of the

(3) We thank Dr. Stephen B. Roeder for the loan of this unit.
(4) D. W. G. Smith and J. G. Powles, *Mol. Phys.*, **10**, 451 (1966).
(5) A. Allerhand, *Rev. Sci. Instrum.*, in press. We thank the author for a preprint of this work, which discusses errors in T_2 measurements produced by field fluctuations.
(6) A. Berger, A. Loewenstein, and S. Meiboom, *J. Amer. Chem. Soc.*, **81**, 62 (1959).
(7) R. L. Vold and A. Correa, *J. Phys. Chem.*, 74, 2674 (1970).
(8) H. Y. Carr and E. M. Purcell, *Phys. Rev.*, **94**, 630 (1954).

Table I. Protolysis Rates in Acidic Solutions of Aqueous Urea. Steady-State Nmr Measurements

pH	$W_{1/2}$[a]	Log k_{uw}	pH	$W_{1/2}$	Log k_{uw}	pH	$W_{1/2}$	Log k_{uw}
10.0 *M* urea			6.5 *M* urea			3.0 *M* urea		
3.50	4.93	4.10	3.75	6.26	3.60	3.24	4.30	3.63
3.89	7.86	3.55	4.01	9.48	3.31	3.30	4.08	3.69
3.90	7.00	3.67	4.20	12.7	3.10	3.58	5.41	3.32
4.00	8.36	3.51	4.41	15.5	2.96	3.60	5.74	3.36
4.10	9.35	3.42	4.62	21.3	2.64	3.74	5.52	3.25
4.21	11.5	3.30	4.81	22.8	2.52	3.85	7.92	3.07
4.25	11.3	3.31	5.10	18.0	2.23	3.88	8.20	3.04
4.30	13.5	3.21	5.27	13.1	2.02	3.97	8.19	3.04
4.40	15.5	3.01	5.74	5.91	1.49	4.05	9.87	2.79
4.51	19.0	2.97	6.10	3.85	1.09	4.07	9.64	2.84
4.57	18.1	3.00				4.10	9.09	2.92
4.65	23.0	2.83	5.0 *M* urea			4.13	9.56	2.85
4.82	31.2	2.60	3.55	5.50	3.61	4.17	9.68	2.46
4.88	30.3	2.63	3.77	7.55	3.36	4.21	9.67	2.46
5.05	37.1	2.44	3.90	8.77	3.25	4.34	9.76	2.48
			4.00	10.2	3.14	4.38	9.22	2.39
8.0 *M* urea			4.10	11.3	3.04	4.40	9.77	2.48
3.59	5.82	3.75	4.20	13.0	2.94	4.46	8.87	2.35
3.87	8.28	3.27	4.27	14.2	2.85	4.47	9.98	2.52
4.26	14.7	3.04	4.40	16.1	2.68	4.54	7.28	2.16
4.54	27.2	2.74	4.60	16.5	2.57	4.69	6.75	2.00
4.60	21.7	2.76	4.84	13.4	2.27	4.87	6.13	2.02
4.62	23.9	2.67	5.17	7.35	1.97	5.05	4.06	1.67
4.73	27.5	2.41	5.29	7.14	1.83	5.25	3.98	1.61
4.82	26.9	2.55	5.41	5.94	1.71			
4.86	30.6	2.41	5.44	5.60	1.66	2.9 *M* urea		
5.05	27.4	2.41				3.54	5.37	3.25
5.14	25.4	2.26				3.74	5.43	3.24
5.25	21.3	2.15				4.01	7.27	2.91
5.28	21.2	2.19				4.30	7.80	2.57
5.44	16.1	2.03				4.51	7.38	2.43
5.53	13.1	1.94				4.77	4.85	2.12
5.65	9.25	1.77				4.96	5.43	2.11
5.79	8.36	1.76						
5.90	7.47	1.70						

[a] $W_{1/2}$'s are measured values of the full width in hertz of the water resonance at half maximum intensity.

Table II. Protolysis Rates in Basic Solutions of Aqueous Urea. Steady-State Nmr Measurements

pH	$W_{1/2}$	Log k_{uw}	pH	$W_{1/2}$	Log k_{uw}
10.0 *M* urea			5.0 *M* urea		
9.33	3.70	0.93	7.78	2.79	0.41
9.73	7.67	1.42	9.20	4.33	1.52
9.95	9.20	1.53	9.73	11.0	2.17
10.06	10.07	1.61	9.93	14.2	2.39
10.36	31.3	2.15	10.23	16.1	2.61
10.67	39.0	2.33	10.51	12.6	2.90
11.22	20.0	2.92	10.74	9.50	3.15
11.80	8.93	3.43	10.96	7.38	3.35
			11.30	4.86	3.62
8.0 *M* urea			11.60	3.33	4.11
9.05	3.04	0.96			
9.42	5.58	1.42	3.0 *M* urea		
9.88	15.2	1.99	9.20	4.08	1.80
10.24	26.9	2.49	9.77	8.37	2.36
10.69	24.5	2.64	10.13	9.85	2.65
11.28	11.1	3.23	10.43	8.02	2.99
11.73	6.18	3.61	10.84	4.73	3.44
12.00	3.90	4.04	11.25	3.21	3.85
			6.5 *M* urea		
9.14	3.97	1.27	10.58	19.4	2.72
9.50	6.96	1.64	11.02	10.5	3.21
9.99	17.0	2.24	11.33	6.90	3.47
10.35	27.5	2.52	11.66	4.78	3.73
			12.04	3.32	4.12

recursion matrix[9]

$$E = \exp(\mathbf{A}\tau)\exp(2\mathbf{A}^*\tau)\exp(\mathbf{A}\tau) \quad (7)$$

The four by four complex, nonhermitian matrix **A** is given explicitly elsewhere;[7] the asterisk means complex conjugate.

In fitting the experimental data to eq 1–7, the parameters were chosen as follows. p_u and p_w were calculated from sample composition. The chemical shift was found to be 70 ± 1 Hz at 60 MHz, independent of urea concentration.[10] Possible dependence of the chemical shift upon pH was neglected in view of the low concentration of acid or base. $\mathcal{A}/2\pi$ was taken to be 63.4 Hz from measurements of ^{15}N-substituted urea.[11] k_d was arbitrarily taken to be zero. Since the urea resonance consisted of a single Lorentzian line, $\mathcal{A} < (R_1 + k_d)$. In this case, only the sum $(R_1 + k_d)$ remains in eq 4 and these two parameters cannot be obtained separately from measurements of the proton nmr spectrum. Numerical calculations indicate that a similar result holds for the spin-echo relaxation curves. Thus the values of R_1 reported here are upper limits only, but the chemical exchange rates k_{uw} are not affected by this ambiguity. The parameters k_{uw}, R_1, and R_2^0 were varied as described below.

Steady-State Results. For many spectra the full widths at one-fourth, one-half, and three-fourths maximum intensity of both water and urea peaks were measured, as well as the urea–water peak separation and the ratio of the urea peak height to the central minimum. All of these parameters are sensitive to the exchange parameters and, in differing degree, to instrumental errors.[12] Because of the low integrated intensity and large width of the urea peak, neither peak separation nor peak-to-valley ratio could be used to estimate kinetic parameters with precision. All kinetic results reported here are based on the line widths. Since the observed resonances are Lorentzian over all but a narrow pH range corresponding to partial coalescence, only the full widths at one-half maximun intensity are reported (see Tables I and II).

The urea line widths could be measured accurately only in neutral solution corresponding to negligible exchange broadening of the water resonance. Equations 1–6 were used to estimate values of the nitrogen quadrupole relaxation rate R_1 for zero chemical exchange rate, which matched the observed urea line widths. In these and all other line-shape calculations, R_2^0 was taken as 6.28 rad/sec from the line width of a completely collapsed spectrum (at pH about 0) for each urea concentration. The upper limits to R_1 obtained in this way are shown in Figure 1.

Using appropriate values of R_1 from Figure 1 and values of the other parameters described above, eq 1–6 were

(9) H. S. Gutowsky, R. L. Vold, and E. J. Wells, *J. Chem. Phys.*, **43**, 4107 (1965).

(10) The chemical shift was measured in solutions containing about 1% dioxane and acetone as internal standards, at neutral pH where the water line showed no exchange broadening. The urea–water shift is accurate to ±1 Hz because the urea line is quite wide. The dependence of the acetone–urea shift upon urea concentration is linear within experimental error and yields a molal shift of urea of not more than 0.002 ppm. The acetone–dioxane shift was independent of urea concentration.

(11) J. B. Lambert, B. Roberts, G. Binsch, and J. D. Roberts, "Nuclear Magnetic Resonance in Chemistry," B. Pesce, Ed., Academic Press, New York, N. Y., 1965, p 272.

(12) A. Allerhand, J. Jonas, H. S. Gutowsky, and R. M. Meinzer, *J. Amer. Chem. Soc.*, **88**, 3185 (1966).

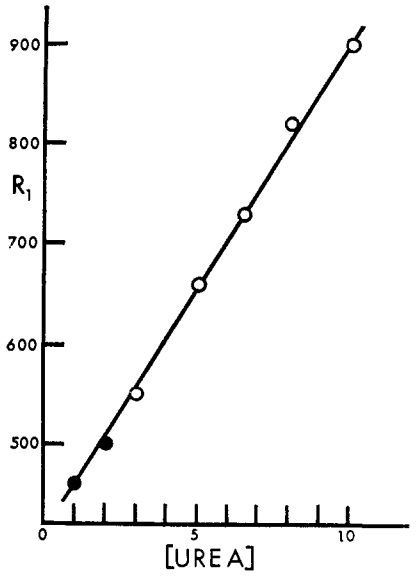

Figure 1. Nitrogen spin-lattice relaxation rates in sec^{-1} as a function of urea concentration. The values of R_1 are upper limits only. See text for details.

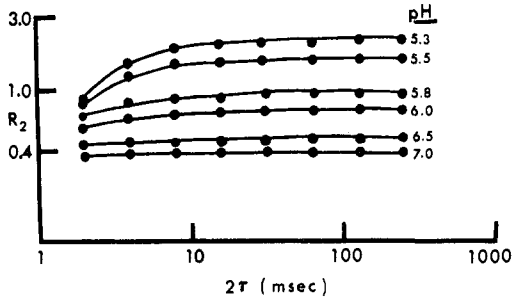

Figure 2. Relaxation curves for 1 M urea in acidic solution. R_2 is the apparent transverse relaxation rate corrected as described in the text for diffusion. 2τ is the 180° pulse spacing in msec.

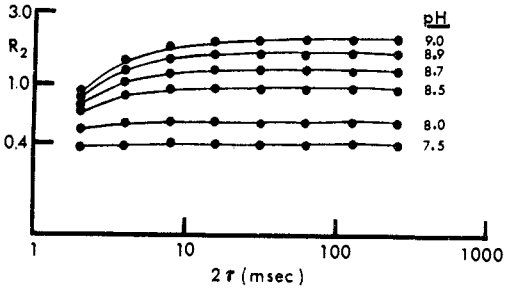

Figure 3. Relaxation curves for 1 M urea in basic solutions.

used to obtain graphs of the line width, $W_{1/2}$, as a function of k_{uw}, the inverse lifetime of a proton on urea. For each urea concentration the appropriate graph was used with the experimental values of $W_{1/2}$ to estimate k_{uw} as a function of pH. Values obtained in this way are shown in Tables I and II.

Spin-Echo Results. Relaxation curves for 1 and 2 M urea are shown in Figures 2–5. The points are measured relaxation rates, corrected as described above for diffusion effects. The solid lines were calculated as follows. R_2^0 was taken to be the measured proton spin–lattice relaxation rate for each sample.[13] Using

(13) These relaxation rates were all close to 0.33 sec^{-1} and independent of pH within experimental error. The value of R_2^0 used for steady measurements is 20 times larger, in part because those samples were not degassed, but mostly because of radiation damping in the small single coil of the Varian T-60 probe.

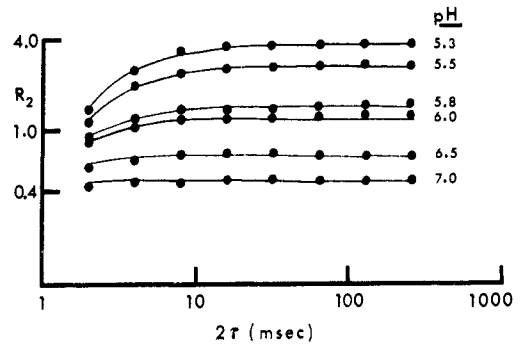

Figure 4. Relaxation curves for 2 M urea in acidic solutions.

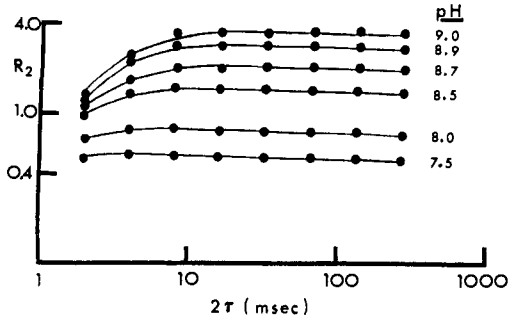

Figure 5. Relaxation curves for 2 M urea in basic solutions.

an algorithm based on eq 7, a large number of relaxation curves were calculated for various values of R_1 and k_{uw}. As expected, the large pulse-spacing limit was sensitive only to the value chosen for k_{uw}, and the sloping region of the relaxation curve depended on the choice of R_1. The solid lines in the figures are those calculated curves with minimum squared deviations from the experimental points. Values obtained for k_{uw} are listed in Table III and for R_1 in Figure 1.

Table III. Protolysis Rates in Aqueous Urea. Spin-Echo Results

pH	k_{uw}, sec^{-1} 2 M urea	k_{uw}, sec^{-1} 1 M urea
5.30	45.38	47.11
5.40	37.82	41.88
5.50	31.51	32.72
5.80	14.50	15.70
6.00	11.98	11.47
6.50	4.79	4.45
7.00	1.89	1.83
7.50	3.15	2.88
8.00	6.81	7.07
8.50	17.02	17.54
8.70	25.84	26.17
8.90	39.08	36.64
9.00	47.90	47.11

Kinetic Analysis. Because high urea concentrations were used, the rates must be analyzed in terms of activities rather than concentrations. The ionic species are present in low enough concentration to ignore ac-

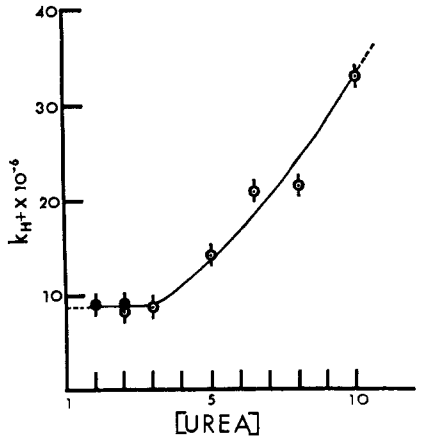

Figure 6. Rate "constant" for acid-catalyzed protolysis in M^{-1} sec^{-1} as a function of urea concentration.

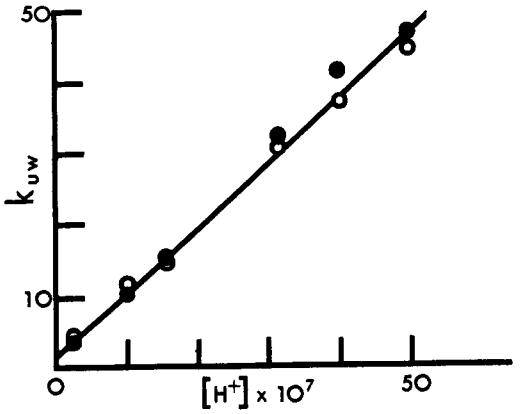

Figure 7. Spin-echo values for the inverse lifetime of a proton on urea in acidic solutions: ●, 1 M urea; ○, 2 M urea.

tivity coefficients, but this is not the case for urea and water. k_{uw} is a pseudo-zero-order rate and is independent of urea activity, assuming first-order dependence of the protolysis rate dA_u/dt upon urea activity. If this rate is also first order in acid, base, and water activity, we obtain

$$k_{uw} = (1/A_u)dA_u/dt = k_{H^+}A_H^* + k_{OH^-}A_{OH^-} + k_{H_2O}A_{H_2O} \quad (8)$$

The first term of eq 8 may be tested with the data of Table I. Figure 6 shows values of k_{H^+} obtained by summing the first and third entries in Table I for each urea concentration, averaged over the set of pH values. The vertical bars represent standard deviations from the average values. The open circles in Figure 6 were obtained from the spin-echo data of Table III. Figure 7 is a plot of spin-echo values of k_{uw} *vs.* hydrogen ion concentration. From this figure we obtain $k_{H_2O}A_{H_2O} \cong 1.5$ sec^{-1}, and from Figure 6, $k_{H^+} = (9.0 \pm 1) \times 10^6$ M^{-1} sec^{-1}. Above about 3 M urea, acid catalysis is not first order in urea activity, although it remains first order in acid.

Testing the second term of eq 8 is more complicated, because the hydroxide activity was not measured directly. For solutions basic enough that the first and third terms of eq 8 are negligible, we have

$$\log k_{uw} - \text{pH} = \log k_{OH^-} + \log K_w A_{H_2O} \quad (9)$$

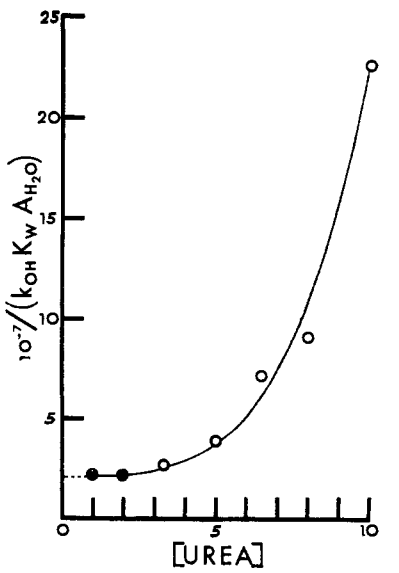

Figure 8. Rate "constant" for base-catalyzed protolysis as a function of urea concentration. Terms K_w and A_{H_2O} involving dissociation of water can be separated from the rate constant k_{OH^-} in M^{-1} sec^{-1} only by extrapolation to zero urea concentration. See text for details.

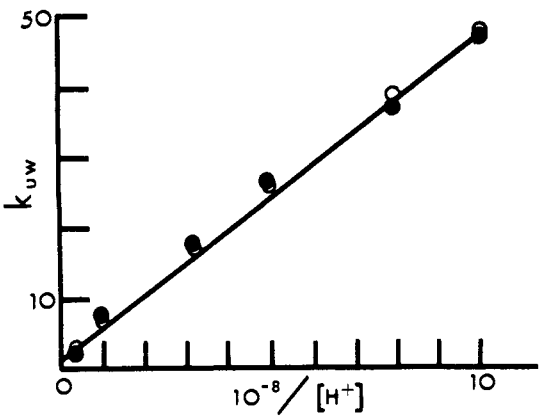

Figure 9. Spin-echo values for the inverse lifetime of a proton on urea in basic solutions: ●, 1 M urea; ○, 2 M urea.

where K_w is the dissociation constant of water in units of moles/liter and A_{H_2O} is the activity of water. Both of these quantities vary with urea concentration. Nevertheless, for any given urea concentration eq 9 predicts that the difference between the first and third entries in Table II is constant. The average values of these differences, with standard deviations, are shown in Figure 8. The open circles were obtained from the slopes of Figure 9, which is a plot of k_{uw} *vs.* inverse hydrogen ion concentration, using spin-echo data of Table III. Figure 9 yields, from the intercept, $k_{H_2O} \cong 1$ sec^{-1}, in good agreement with Figure 7. If Figure 8 is extrapolated to zero urea concentration, the last term of eq 9 may be obtained from the ionization constant of pure water at 32°, 2.1×10^{-14} M^{-2}. In this way we find $k_{OH^-} = (2.4 \pm 1) \times 10^6$ M^{-1} sec^{-1}. In basic solution the protolysis rate departs from first order in urea activity above about 2 M urea.

Discussion

The use of eq 1–7 to obtain protolysis rates involves two important assumptions.[14] Internal rotation about

the C–N bonds may be hindered, rendering the description of protolysis by a single rate k_{uw} inexact. The urea line shape was observed to consist of a single Lorentzian curve and the relaxation curves showed no intermediate plateaus. Hence the rate of internal rotation must be fast compared with fine structure splittings of the urea protons. Part of the urea line width could still be due to incomplete averaging of this fine structure. Calculations discussed elsewhere[7] indicate that for reasonable values of the chemical shift between *urea* protons, only about 10% of the observed urea line broadening could be due to this source; also, the water line widths, upon which all our rates are based, accurately represent the average rate of exchange of all urea protons. Moreover, the chemical shift between urea protons in dimethyl sulfoxide at low temperatures where internal rotation is "frozen out" is unobservably small, even at 100 MHz.[15] We conclude that neglect of hindered internal rotation introduces no significant error.

The other important assumption is an implicit neglect of proton exchange between different aggregated species of urea, which are most likely present in the range of urea concentrations used. In order for such processes to contribute line broadening not described by eq 1–7, there must be appreciable chemical shifts between urea protons in different aggregates. In that case, there would be an appreciable dependence of the urea resonance frequency upon urea concentration. Relative to a double internal standard of dioxane and acetone, the molar shift of aqueous urea is only 0.002 ppm between 1 and 10 *M* urea. Therefore, the rates derived from eq 1–7 may be regarded as average rates for all urea protons.

The inverse lifetime k_{uw} is proportional to hydrogen ion concentration in acidic solutions and inversely proportional to hydrogen ion concentration in basic solution over the entire range of urea concentrations from 1 to 10 *M* urea. In both acidic and basic solutions, k_{uw} depends on urea concentration above about 2 or 3 *M* urea (see Figures 6 and 8). This effect is most likely due to formation of aggregated species of urea with some alteration of solution "structure." As is usual[16] with experiments other than X-ray, neutron, or electron diffraction, these nmr results cannot be used to derive conclusive, detailed descriptions of the structure in aqueous urea. A number of equally plausible speculations concerning the nature of the aggregates can explain the qualitative features of Figures 6 and 8. The only firm conclusion to be drawn from these figures is that aggregated species of urea do affect protolysis rates at urea concentration above about 2 or 3 *M*.

Reversible denaturation of proteins in aqueous urea is thought to be due in part to the formation of mixed clusters of urea and water molecules, permitting nonpolar protein side chains to unwind into more or less vacant regions of solution. This idea, and supporting experimental evidence, has been summarized by Jencks.[17] It may be relevant that the protolysis rate k_{uw} departs from zero order in urea at about the same urea concentrations where urea becomes effective as a protein denaturant.

If urea–water clusters have more than a transient existence in solution they would probably lengthen the correlation time for molecular reorientation of urea molecules, thus increasing the nitrogen relaxation rate. Our derived values of R_1 do increase with increasing urea concentration. However, these values of R_1 are upper limits only and may well contain a contribution from direct proton exchange between urea molecules, which would also increase with increasing urea concentration. Direct measurement of ^{14}N resonance is required to eliminate this complication.

Compared with rate constants for acid-catalyzed protolysis of most amides,[18] k_{H^+} for urea is anomalously large by about four orders of magnitude. This is most likely due to a different protolysis mechanism. Rate constants for bimolecular collision of OH^- with amide nitrogen atoms are usually on the order of 10^6. Low rate constants for acid-catalyzed protolysis are thought[6] to be due to a low probability of collision of H_3O^+ with nitrogen, most proton transfers occurring at the carbonyl oxygen and not contributing to the observed line shape. The large value of k_{H^+} for dilute aqueous urea thus indicates protonation on urea nitrogen rather than oxygen. This is the reverse of what is observed in "super acid" urea solutions.[15]

Conclusion

Protolysis rates have been measured in acidic and basic urea solutions from 1 to 10 *M* in urea. Protonation occurs at urea nitrogen rather than oxygen. Above about 2 or 3 *M* urea, formation of aggregated species of urea alters the form of the rate law. No more detailed picture of the structure of the aggregates can be obtained from the nmr rate data presented here.

(14) Equations 1–7 also involve neglect of nonsecular parts of the proton–nitrogen scalar coupling. Since the largest measured exchange rate is about 10^4, and the proton–nitrogen chemical shift is about 10^8, little error is made.

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