General Base Catalyzed Proton Exchange in Amides

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The kinetics of general base catalyzed proton exchange in *N,N'*-dimethylurea and a series of secondary amides were studied. Kinetic data were determined by NMR line-shape analysis. A good linear correlation was observed for nine amides by plotting $\log k_{OH}$ for $RCONHCH_3$ vs. pK_a of RCOOH. For amides with N–H more acidic than water ($pK_a<15.7$), general base catalysis is observed, the Brønsted β is unity, and the reaction with hydroxide is diffusion-controlled. It is concluded that general base catalysis is inoperative in proteins or polypeptides.

Proton transfers[1] are among the most fundamental of chemical reactions. During recent years, proton exchange in amides, including ureas, has been of considerable interest due primarily to its capability to provide information to questions of biochemical significance.[2—13] Besides the acid catalyses, there is one more mechanism for proton exchange in amides. It has been found that base can catalyze the proton exchange, via an imidate intermediate.[14,15] There is no controversy about this base catalysis mechanism. As shown in Eq. 1, the base removes the amide proton to generate an imidate, and then the imidate anion can simply pick up a proton from solvent or any other proton source to complete the proton exchange.

$$\mathrm{RCNHR'} + \mathrm{B} \underset{k_r}{\overset{k_B}{\rightleftharpoons}} \mathrm{RCO{=}NR'^{-}} + \mathrm{BH^+} \qquad (1)$$

Although the base catalysis is mechanistically straightforward, it is still remarkable because of the information that can be adduced.

General base catalysis has been reported by Klotz et al.,[16] who found that proton exchange of *N*-methylacetamide was catalyzed by hydroxylamine in aqueous solutions. Krauss and Cowburn have reported that base catalysis of the amide proton of the tyrosine residue in oxytocin is 5 times enchanced relative to that in the random-coil peptide of identical amino acid sequence.[17] They attributed this effect to the *N*-terminal amino group, which is hydrogen bonded with the carbonyl group of tyrosine. The proton on the nitrogen atom is therefore more susceptible to be abstracted by base.

For amides with a nitrogen proton more acidic than water ($pK_a=15.7$), the proton transfer from water to the corresponding imidate, which is the reverse process of Eq. 1, is energetically unfavorable and therefore, is slower than the diffusion-controlled rate.[18] In contrast, the proton transfer from general acid to the imidate is energetically favorable and diffusion-controlled, so the contribution of this process should be significant relative to the contribution of proton transfer from water to imidate as long as the concentration of general acid is high enough. This specific base-general acid catalysis is kinetically equivalent to a general base catalysis in the forward direction. From the satisfaction of the microscopic reversibility, the exchange rate catalyzed by general base B should be significant relative to that catalyzed by specific base OH^-. In order to observe general base catalysis experimentally, the condition must be controlled at lower pH to suppress the specific base catalysis. The rate constant k_B can be represented as Eq. 2,

$$k_B = k_r \frac{K_a^{AH}}{K_a^{BH}} \qquad (2)$$

where k_r is a diffusion-controlled rate constant, and K_a^{AH} and K_a^{BH} are dissociation constants of amide and general acid, respectively. Since k_r is a diffusion-controlled rate constant, it should be independent of acidity for different conjugate acids BH^+ with K_a^{BH} greater than K_a^{AH}.[18] Consequently, k_B is inversely proportional to K_a^{BH} and should give a unity β in the Brønsted plot of $\log k_B$ vs. pK_a^{BH}.[19]

In contrast, for an amide with a nitrogen proton less acidic than water, the reprotonation of the corresponding imidate in aqueous solution is diffusion-controlled. No general base catalysis is expected for this amide because proton transfer to the imidate from water or from general acid is diffusion-controlled.

All of our studies were done on secondary amides, $RCONHCH_3$ with different substituents R, in order to elucidate the substituent effect on this general base catalyzed proton exchange. These compounds have the advantage that the exchange rate can be measured reliably by line-shape analysis of the *N*-methyl doublet.

Experimental

Chemicals and Sample Preparation. Amides and *N,N'*-dimethylurea were either commercially available or synthesized by literature methods. Exchange sample solutions were prepared by dissolving the same amount of sample in buffer solutions with different concentrations of buffer. The range of buffer concentrations was varied up to about 10 fold. Sodium chloride was added to maintain constant ionic strength in the sample solutions. Typical procedure of sample preparation is described in the following with formate buffered *N*-methyltrifluoroacetamide. To a 10 mL volumetric flask, neat *N*-methyltrifluoroacetamide (0.3 g) and a certain amount of concentrated formate buffer ($[HCOOH]/[HCOO^-]=0.15$ M/0.85 M) were added (1 M=1

mol dm^{-3}). Then this solution was diluted to the mark of 10 mL with NaCl solution (0.85 M). By varying the mixing proportions of stock buffer and NaCl solution, sample solutions with different buffer concentrations were prepared. [$HCOO^-$] of these samples varied from 0.808 M to 0.085 M. Small pH discrepancies between sample solutions with different buffer concentrations were adjusted by adding microliter quantities of hydrochloric acid and/or sodium hydroxide solution. The pH was 4.46 and the ionic strength was 0.82 for these sample solutions.

pH Measurement. pH measurements were made with a Corning Model 125 pH meter connected to an Orion combination pH electrode or an Ingold combination pH electrode. The meter was calibrated with standard buffer solutions obtained from Corning and Mallinckrodt. Electrodes were rinsed with deionized water and dried before each measurement. Measured pH values were used to calculate the concentration of OH^-.

Kinetics. Since the *N*-methyl protons of the secondary amides (urea) exhibit coupling to the N–H protons, the proton exchange rate constants can be calculated by the analysis of NMR line-shape of the *N*-methyl doublets. The valley-to-peak intensity ratio of an *N*-methyl doublet was measured. The buffer ratio was chosen to produce a pH so that the valley-to-peak ratio of the *N*-methyl doublet was about 0.5 at the solution with least buffer concentration. This then permitted maximum accuracy in determining the additional rate due to the additional buffer. The pseudo-first-order rate constants were determined from an extended table[20] of these ratios as calculated from the line-shape equation,[21] without neglecting the influence of inherent line width. This method represents a digital equivalent of a total line-shape analysis.[22]

NMR spectra of all the samples were taken on a Varian EM390 90 MHz NMR spectrometer. Samples were allowed to equilibrate for 15 min to the probe temperature of 34 °C, which was measured with an ethylene glycol sample by the method of Becker.[23]

Determination of the pK_a of *N*-Methyltrifluoroacetamide. The pK_a of *N*-methyltrifluoroacetamide was determined by pH measurement. The pH reading was taken by a Corning 125 pH meter equipped with an Orion combination pH electrode at 0 °C. The pH meter was calibrated with pH 7 buffer at room temperature first. Then it was calibrated with 0.100 M sodium hydroxide solution at 0 °C. Both the electrode and 0.100 M sodium hydroxide solution were ice-cooled for at least 15 min before calibration. The pH reading of this sodium hydroxide solution was set to 13.94 since pK_w is 14.94 at 0 °C.[24]

N-methyltrifluoroacetamide (0.254 g, 0.02 mol) was added to a test tube. To another test tube, 10.0 mL of 0.100 M sodium hydroxide solution was added. Both test tubes were sealed with rubber septums and cooled in an ice-bath for 15 min. The sodium hydroxide solution was then poured into the test tube which contained *N*-methyltrifloroacetamide and shaken vigorously. The pH changes of the *N*-methyltrifluoroacetamide solution were followed over a period of 12 min. Experimental data were fitted to a quadratic equation $pH = a + bt + ct^2$, where a, b, c are constants and t is time in second. The pK_a of *N*-methyltrifluoroacetamide at 0 °C was determined by Eq. 3,

$$pK_a = pH + \log \frac{[AH]}{[A^-]} \quad (3)$$

where [AH] and [A^-] are the concentrations of *N*-methyltrifluoroacetamide and the corresponding imidate. Since [AH] is equal to [A^-] and pH is equal to a at zero time, pK_a is equal to pH at zero time according to Eq. 3.

Results

General Base Catalysis Studies of general base catalyzed proton exchange were undertaken through a sequence of secondary amides and *N,N'*-dimethylurea. First-order rate constants were observed from line-shape analysis. Pseudo-first-order rate constant can be expressed as Eq. 4,

$$k_{obsd} = k_{OH}[OH^-] + k_B[B] \quad (4)$$

where k_{OH} is the second-order rate constant of specific base catalysis in $M^{-1}s^{-1}$ and k_B is the second-order general base catalyzed rate constant, also in $M^{-1}s^{-1}$. At constant pH, both k_B and k_{OH} can be determined by plotting k_{obsd} vs. [B]. The results are listed in Table 1. No general base catalysis was observed in amides $RCONHCH_3$ with substituents $R = CH_3NH$, $CH_3NHCOCH_2CH_2$, C_6H_5, CH_3OCH_2, $CH_3CONHCH_2$, H, $CH_3NHCOCH_2$, $NCCH_2$, and CH_2Cl. However, amides with strongly electron-withdrawing substituents R, like *N*-methyl-2,2-dichloroacetamide, *N*-methyltrichloroacetamide and *N*-methyltrifluoroacetamide, did show general base catalysis. The more basic the catalyzing base is, the greater the second-order rate constant k_B is. Amides that show general base catalysis all have k_{OH} close to diffusion-controlled rate constant.

Amides with electron-withdrawing substituents, especially *N*-methyltrifluoroacetamide, although hydrolyzed quickly at high pH, were very stable under the experimental condition. No significant hydrolysis of *N*-methyltrifluoroacetamide was observed after watching the sample solution over a period of one hour.

Determination of pK_a of *N*-Methyltrifluoroacetamide. The hydrolysis rate of *N*-methyltrifluoroacetamide is slow enough to be followed under the experimental condition. The results of pH measurement of the 1:1 *N*-methyltrifluoroacetamide: *N*-methyltrifluoroacetimidate solution against time are fitted to a quadratic equation which is shown as Eq. 5,

$$pH = 13.16 - 6.084 \times 10^{-4}t - 1.326 \times 10^{-7}t^2 \quad (5)$$

where t is the reaction time in second. By extrapolating back to zero time as shown in Fig. 1, or from Eq. 5, the initial pH of the sample solution was 13.16. Since the concentrations of *N*-methyltrifluoroacetamide and the imidate anion were equal in the prepared sample solution, pK_a is equal to pH of the solution at zero time, so that a pK_a value of 13.16 was obtained for *N*-methyltrifluoroacetamide at 0 °C.

Table 1. Rate Constants of General Base and Specific Base Catalyzed Proton Exchange in Amides and Ureas

R	B	pK_a^{BH}	$k_B/M^{-1}s^{-1}$	$k_{OH}/M^{-1}s^{-1}$	$\log k_{OH}$
CH_3NH	NH_3	9.25	0.0±1.9	$(1.40\pm0.23)\times10^6$	6.15
$CH_3NHCOCH_2CH_2$	HPO_4^{2-}	7.21	−0.3±1.1	$(6.46\pm0.54)\times10^7$	6.81
C_6H_5	Imidazole	6.95	−1.7±0.4	$(5.23\pm0.05)\times10^7$	7.72
CH_3OCH_2	HPO_4^{2-}	7.21	−1.9±1.6	$(3.81\pm0.43)\times10^7$	7.58
H	HPO_4^{2-}	7.21	−0.6±2.3	$(7.87\pm0.75)\times10^7$	7.90
$CH_3CONHCH_2$	HPO_4^{2-}	7.21	1.1±2.3	$(5.98\pm0.68)\times10^7$	7.78
$CH_3NHCOCH_2$	HPO_4^{2-}	7.21	−2.1±1.6	$(2.59\pm0.08)\times10^8$	8.41
CH_2Cl	HPO_4^{2-}	7.21	3.6±7.7	$(1.65\pm0.05)\times10^9$	9.22
$NCCH_2$	HPO_4^{2-}	7.21	−1.4±2.3	$(1.27\pm0.03)\times10^9$	9.10
$CHCl_2$	Pyridine	5.25	4.1±0.8	$(7.03\pm0.10)\times10^9$	9.85
CCl_3	CH_3COO^-	4.75	7.1±0.5	$(7.27\pm0.23)\times10^9$	9.86
CF_3	$HCOO^-$	3.75	5.3±0.8	$(1.56\pm0.14)\times10^{10}$	10.19
	CH_2=$CHCOO^-$	4.25	8.8±0.6	$(2.01\pm0.06)\times10^{10}$	10.30
	CH_3COO^-	4.75	40.6±2.8	$(2.01\pm0.25)\times10^{10}$	10.30

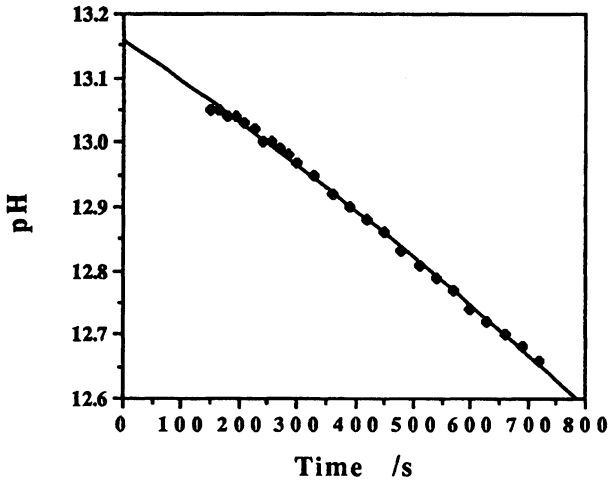

Fig. 1. Plot of pH measurement of 1:1 *N*-methyltrifluoroacetamide *N*-methyltrifluoroacetimidate solution vs. time. When extrapolated back to time zero, pH is equal to 13.16.

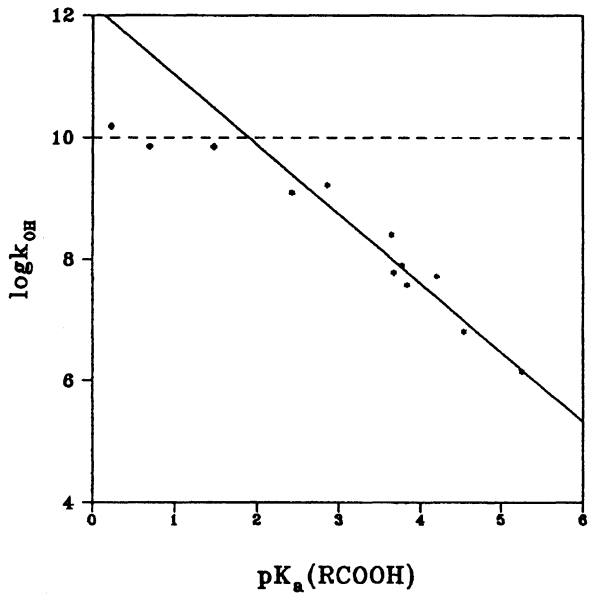

Fig. 2. Correlation between $\log k_{OH}$ for base catalyzed proton exchange in *N*-methylamides $RCONHCH_3$, and the pK_a of the corresponding RCOOH: solid line, best linear fit for nine amides, slope=1.14; dashed line, diffusion-controlled rate constant.

Discussion

Error Analysis. First-order rate constants observed from NMR line-shape analysis are reproducible and the errors of reproducibility are less than 5%. Errors listed in the data tables are standard deviations from the least square fitting. Second-order rate constants generally have a standard deviation of ±5—10%. Therefore, the general base catalysis we have observed are well beyond experimental error.

Base Catalyzed Proton Exchange. The experimental results collected in Table 1 demonstrate that the more electron-withdrawing the substituent R is, the greater the value of k_{OH} is. This is reasonable since the electron-withdrawing groups can increase the acidity of the N–H proton inductively and therefore make it easier to be removed by OH^-.

We have sought a quantitative manner to describe these phenomena caused by substituent effects. A most suitable parameter is the pK_a of the corresponding carboxylic acid RCOOH which has been utilized successfully by Charton[25] and Perrin.[4] Figure 2 is a plot of $\log k_{OH}$ for $RCONHCH_3$ vs. the pK_a of RCOOH. It can be seen that there is a good correlation for nine substituents R=CH_3NH, $CH_3NHCOCH_2CH_2$, C_6H_5, CH_3OCH_2, $CH_3CONHCH_2$, H, $CH_3NHCOCH_2$, $NCCH_2$, and CH_2Cl (slope=1.14±0.12, $|r|=0.926$). Therefore, the specific base (OH^-) catalyzed proton exchange is quite sensitive to inductive effects in a way that is well described by pK_a of carboxylic acids. The linear correlation in Fig. 2 predicts that the three amides with more electron-withdrawing substituents

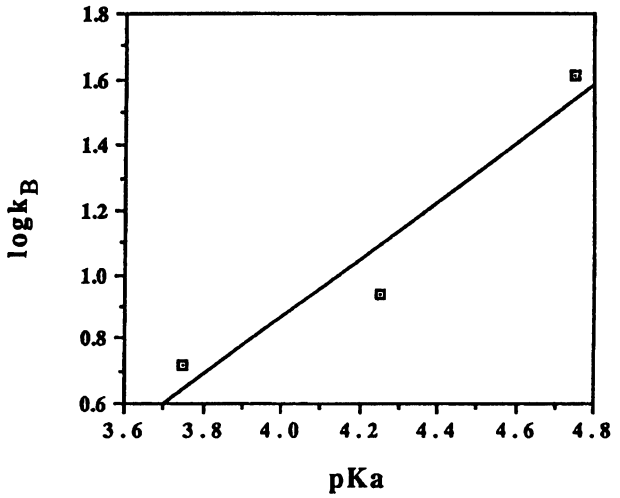

Fig. 3. Brønsted plot of *N*-methyltrifluoroacetamide with $\log k_B$ and pK_a.

($R=CHCl_2$, CCl_3, CF_3) should possess k_{OH} comparable to diffusion-controlled rate constant (10^{10} $M^{-1}s^{-1}$).[18] This is consistant with the experimental observed values as shown in Table 1. We may conclude here that the N–H protons of these three amides are more acidic than water protons.

No general base catalysis was observed for amides with N–H protons less acidic than water protons. As mentioned in the introduction, the reprotonation of the imidates is diffusion-controlled in aqueous solution, so that the observed first-order rate constants are not expected to be affected by changing buffer concentration as long as the pH of sample solutions is kept constant. Amides that are more acidic than water (have diffusion-controlled k_{OH}) do show general base catalysis as expected. The measured pK_a of *N*-methyltrifluoroacetamide at 0 °C is 13.16, which is lower than that of water. Indeed, general base catalysis of *N*-methyltrifluoroacetamide gives a Brønsted β of 0.89±0.25 (see Fig. 3), which is reasonably close to unity.

Since $k_{acetate}$ of *N*-methyltrifluoroacetamide is 40.6 $M^{-1}s^{-1}$ and pK_a of CH_3COOH is 4.75, inputting these values to Eq. 2 gives the k_r of 1.04×10^{10} $M^{-1}s^{-1}$, which is a proper number for a diffusion-controlled rate constant. We can also estimate pK_a of other amides which show general base catalysis from Eq. 2. The pK_a of *N*-methyltrichloroacetamide is estimated to be around 13.9, based on the $k_{acetate}$ value of *N*-methyltrichloroacetamide and an assumption that k_r of *N*-methyltrichloroacetamide is the same as that of *N*-methyltrifluoroacetamide. Similarly, the pK_a of *N*-methyldichloroacetamide is estimated to be about 14.6.

Biochemists have employed proton exchange to probe static and dynamic aspects of polypeptide molecular structure. As the concentration of phosphate in the intracellular fluid is so great (ca. 10^{-3}—10^{-1} M), it might make a significant contribution to proton exchange in polypeptides via general base catalysis.

We therefore, are especially interested in *N*-acetylglycine methylamide ($R=CH_3CONHCH_2$) which is most closely comparable to peptides and proteins. Since there was no general based catalysis observed for *N*-acetylglycine methylamide, it is concluded that general base catalysis cannot exist for polypeptides in the intracellular fluid except those exhibit "neighboring group participation[17]".

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