Catalysis of Amide Hydrolysis and Formation under Neutral Conditions by a Zwitterionic Imidazolium Thiolate

J. W. Keillor, A. A. Neverov, and R. S. Brown*

Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

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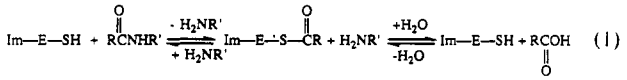
Abstract: As a simple model for the hydrolysis of peptides mediated by the cysteine proteases, 2-(mercaptomethyl)-1-methylimidazole (**1**) was used to catalyze the hydrolysis of four nonactivated amides, namely, formamide, dimethylformamide, *N*-formylmorpholine, and formanilide (**3a–d**), at 98 °C, pD 7.6–8.0, μ = 1.0 (KCl). Progress of the hydrolysis reactions was followed by ^{1}H NMR, and the kinetics as a function of added [**1**] were used to determine the second-order catalytic rate constants (k_{cat}). A putative intermediate *S*-formyl thiolester of **1** (**4**) was not observed to build up during the course of the hydrolysis: the partitioning of authentic **4** between H_2O and morpholine and between H_2O and aniline was determined (98 °C, pD 8.0). The hydrolysis of *N*-formylmorpholine was observed to be catalyzed by added phosphate buffer under the same conditions. When the hydrolysis of a 200 mM D_2O solution of *N*-formylmorpholine was allowed to proceed to completion, an equilibrium position of 33 mM amide, 167 mM HCO_2H, and 167 mM morpholine was attained: that same equilibrium position was obtained starting with a solution 200 mM in each of HCO_2H and morpholine. The conditional equilibrium constant, K'_{eq} = [NFM]/([HCO_2H]$_{tot}$·[morpholine]$_{tot}$), was found to be 1.2 M^{-1}.

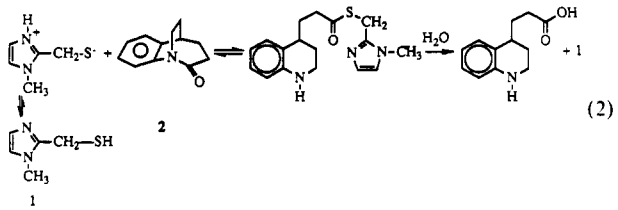
Introduction

The cysteine proteases comprise a large class of enzymes, the active sites of which contain an essential cysteine SH and histidine imidazole unit.[1] The catalytic pathway for hydrolysis of both ester and amide substrates involves formation of a cysteine *S*-acyl enzyme as in eq 1.[2] Although the great bulk of mechanistic studies have concerned the hydrolytic pathway, important investigations reveal that the cysteine proteases (like other classes of proteases) can, under certain circumstances, resynthesize amides from their constituent carboxylic acids and amines.[3] The protein resynthesis, being the microscopic reverse of hydrolysis, also proceeds through the cysteine S-acyl intermediate. Thus, the propensity of the *S*-acyl intermediate to undergo hydrolysis or aminolysis depends in part upon its partitioning between attack by H_2O or amine attack.



(1)

As part of a long term-program to provide small molecule mimics of the cysteine proteases, we have synthesized thiol imidazole **1** and evaluated its mechanism of reaction with an ester (*p*-nitrophenylacetate)[4] and the activated anilide **2**.[5] Those studies indicated that nucleophilic attack of the zwitterionic forms of **1** on **2** generates the corresponding thiolester, which subsequently undergoes hydrolysis with intramolecular general base assistance by the pendant imidazole. Reported herein is the propensity of **1** to act as a catalyst for the hydrolysis of the nonactivated amides formamide, dimethylformamide (DMF), N-formylmorpholine (NFM), and formanilide (FA), amides **3a-d** respectively. Also reported is the use of **1** as a catalyst of the reverse reaction, namely, the re-formation of NFM from formic acid and morpholine in D_2O at near neutral pD values. Finally, we have measured the partitioning of thiolester **4** between attack by H_2O and aniline or morpholine under conditions comparable to those used for the catalyzed hydrolysis of the amides.



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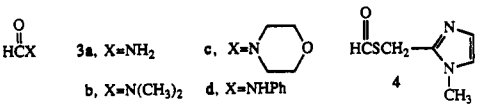

Experimental Section

General. High-field NMR spectra were recorded on a Bruker WH-200 (200 MHz) spectrometer.

Materials. Formamide (**3a**), *N*,*N*-dimethylformamide (DMF, **3b**), *N*-formylmorpholine (NFM, **3c**), and sodium 3-(trimethylsilyl)propanesulfonate (DSS) were commercially available (Aldrich) and were used without further purification. Formanilide was prepared by the procedure of Deslongchamps et al.[6] Phosphate buffer (KH_2PO_4/K_2HPO_4) was reagent grade (Sigma) and was used as supplied. Deuterium oxide (99.9%,

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Table 1. Rate Constants for the Hydrolysis (and Formation) of Various Formamides Mediated by Thiol Imidazole **1** in D_2O Solution (T = 98 °C, μ = 1.0 (KCl))

amide	k_2^{obsd} (M^{-1} s^{-1})[a] × 10^5	k_0^{obsd} (s^{-1})[e] × 10^8
3a (formamide)	13 ± 1[b]	36.4 ± 4.2[g]
3b (DMF)	1.6 ± 0.2[c]	2.8 ± 0.6[g]
3c (NFM)	1.1 ± 0.1[d]	3.0 ± 0.1[c]
3d (formanilide)	5.5 ± 0.5[c]	76 ± 10

[a] The second-order rate constants are the slopes of the linear least squares fits of the plots of the pseudo-first-order rate constants for hydrolysis vs four concentrations of **1**, determined in duplicate. The relative error of these values is estimated at 10%. $k_2^{obsd} = k_{cat}$ in Scheme 1 and text. [b] pD 7.8. [c] pD 8.0. [d] k_{cat} of Scheme 1, pD 8.0. [e] Given as the intercept of plots k_{obsd} vs [**1**] or as observed pseudo-first-order rate constants measured by initial rate methods with no added catalyst.

Aldrich) was purged of O_2 by passage of a stream of Ar through it for several hours. 2-(Mercaptomethyl)-*N*-methylimidazole (**1**) was prepared as reported.[4b]

2-((Formylthio)methyl)-*N*-methylimidazole (4). This was prepared from the reaction of **1**[4b] and formyl fluoride[7] as follows. To a 10-mL solution of 1.3 g of **1** (7.92 mM) in dry CH_3CN was added 1.1 mL (7.92 mmol) of triethylamine. Gaseous formyl fluoride[7] was introduced into the solution while the temperature was maintained at 0 °C with an ice bath. During the addition a white precipitate appeared. After 30 min, the mixture was allowed to come to room temperature, after which time it was stirred for another 30 min. The mixture was then cooled to −30 °C and filtered, and the excess solvent was removed *in vacuo* at room temperature. The material prepared in this way unavoidably contained ~7% of formic acid (by NMR), and it was stored at −78 °C to avoid decomposition: ^{1}H-NMR (CD_3CN) δ 10.23 (s, 1 H), 7.21 (s, 2 H), 4.51 (s, 2 H), 3.73 (s, 3 H); IR[8] (film) 1673.2, 761.7 cm^{-1}; exact mass calcd for $C_6H_8N_2OS$ 156.0357, found 156.0360.

Kinetics. In a typical kinetics experiment, a solution that was 100 mM (for DMF or formamide) or 200 mM (for NFM) in amide, 0–150 mM in **1**, and 35 mM in sodium 3-(trimethylsilyl)propanesulfonate (DSS) as a ^{1}H-NMR standard was prepared in 6–10 mL of D_2O at pD 7.8–8.0 (buffered by **1** itself) with the ionic strength controlled by KCl such that μ = 1.0. Aliquots of this solution 0.6 mL in volume were then transferred into each of eight argon-purged 5-mm NMR tubes, which were flame-sealed. ^{1}H-NMR spectroscopy was performed on two of the tubes for the "zero time" spectra. All of the tubes were then heated to 98 °C (boiling water vapor), and at various times, duplicate tubes were removed and frozen. The ^{1}H-NMR spectra of all eight tubes were subsequently recorded, and the ^{1}H-NMR signals due to the formate H (δ 8.5) and formyl H (δ 8.0) were integrated relative to DSS to determine the progress of the hydrolysis reaction. The solution pD was measured before and after the reactions with a Radiometer VIT90 titrator equipped with a Radiometer GK2401C combination electrode and did not change by more than 0.05 units; even in the case of the unbuffered blank reactions, the solution pD varied by no more than 0.05 units.

For formamide, the hydrolysis was sufficiently fast that a nonlinear least squares (NLLSQ) fit of the exponential decay of formamide concentration with time was possible, giving a pseudo-first-order rate constant for each concentration of **1**. However, for the slower hydrolyses of DMF and NFM, the initial rates of the reactions were determined by measuring, by linear least squares methods, the slopes of the first 6–10 points on the plots of amide or HCO_2H concentration versus time for the initial 3–10% of the reaction (up to 14 days required for the blank runs) and dividing by the initial amide concentration. This was necessary as the catalyst appeared to begin to decompose after ~24 h to what is believed to be a product of oxidation or hydrolysis. The second-order rate constants are compiled in Table 1; in Tables 1S–4S (supplementary material) are the pseudo-first-order rate constants for the hydrolyses of **3a–d** at various [**1**].

Formation Reactions. The formation of NFM was similarly followed by ^{1}H-NMR in D_2O, pD 8.0, μ = 1.0 (KCl), using solutions 0 or 100 mM in **1**, or 200 mM in phosphate, and 200 or 400 mM in both formic acid and morpholine. The observed pseudo-first-order rate constants were determined by dividing the initial slope (three to five points, in duplicate) of the plot of [NFM] vs time by the initial concentration of formic acid.

(7) Olah, G. A.; Kuhn, S. J. *J. Am. Chem. Soc.* **1960**, *82*, 2380.

(8) Olah and Kuhn (ref 7) report that a variety of alkyl thiolformates show strong C=O stretches at ~1681 cm^{-1} and a C–S stretching band at 755–763 cm^{-1}.

For the blank reaction, using 200 mM in each component, seven days were required to generate an observed amide concentration of 10 mM. However, it is important to realize that the initial rate of the formation process cannot be directly compared with the initial rate of hydrolysis of **3c** since the composition of the solutions with respect to formate and amine concentrations is different in each case.

Equilibrium End Point Determination. Two sealed NMR tubes 200 mM in each of formic acid, morpholine, and phosphate (pD 7.6, μ = 1.0 (KCl)) were heated for 20 days at 98 °C. Periodically, the tubes were removed from heating, cooled, and examined using ^{1}H-NMR. Nonlinear least squares analysis of the eight data points of [NFM] vs time so collected gave a pseudo-first order rate constant for formation of NFM with phosphate catalysis. The hydrolysis of 200 mM NFM in the presence of 200 mM phosphate was monitored in an identical manner to give a pseudo-first order rate constant for the hydrolysis of NFM with phosphate catalysis. The final, asymptotic value of [NFM] from the exponential fit for both of these experiments was identical within experimental error.

Solvent Isotope Effect Studies. The equilibrium end point experiment described above was repeated in H_2O (pH 7.6, μ = 1.0 (KCl)) using 5% D_2O as a lock for the NMR analysis.

Partitioning of 2-((Formylthio)methyl)-*N*-methylimidazole (4) between D_2O and Amines. Product analyses for reaction of formyl thiolester **4** with D_2O and morpholine or aniline were conducted by ^{1}H-NMR after reacting in D_2O solutions at pD 8.0, μ = 1.0 KCl, T = 98 °C, containing 0.5 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) as a buffer. In two sets of experiments, 25 μL of freshly prepared thiolester (0.1 M in CD_3CN) was injected into an NMR tube containing the D_2O solution of buffer and 1.5×10^{-2} or 4.5×10^{-2} M aniline. The NMR tubes had been preheated to 98 °C in a water bath prior to the addition, and they were kept there for 10 min following the addition. The ^{1}H-NMR spectrum was then recorded, and the signals for formate (δ 8.5) and formanilide (δ 8.27, 8.66) were integrated. The integrated intensity of the formate proton was corrected for the amount of contaminating formic acid that was originally present in the sample of thiolester.

In another set of experiments, the deacylation of the thiolester was conducted in a similar fashion but the NMR tubes contained 0.1 M aniline and 1.5×10^{-2} M morpholine. After reaction, NMR integration of the N-formylmorpholine (δ 8.1) and formanilide (δ 8.27, 8.66) gave the relative partitioning of the thiolester between the two amines.

Results

Kinetics. The kinetics of hydrolysis of **3a–d** mediated by varying [**1**] (0–200 mM, T = 98 °C, pD 7.8–8.0, μ = 1.0 (KCl)) was investigated by ^{1}H-NMR. The signals due to the formate-H (δ 8.5) and formyl H (δ 8.0) for **3a–c** (δ 8.3 and 8.7 for **3d**) were integrated relative to sodium 3-(trimethylsilyl)propanesulfonate (DSS) to determine the progress of the hydrolysis for times up to 24 h. No accumulation of formyl intermediates (such as thiolester **4**) was observed under any conditions. However, at long reaction times, there appears to be some decomposition of the catalyst (**1**) as evidenced by the formation of a new N–CH_3 peak at ~δ 3.70. The exact nature of the decomposition product is unknown, but could be surmised to be a product of thiol oxidation (e.g., disulfide) or hydrolysis (e.g., 2-(hydroxymethyl)-*N*-methylimidazole). Because of the problems of long-term catalyst stability, the kinetic data were obtained only for times where catalyst decomposition was less than 10%.

The pseudo-first-order rate constants for the hydrolysis of **3a–d** were plotted against [**1**] thereby giving the second-order catalytic rate constants at 98 °C reported in Table 1. The observed initial pseudo-first order rate constants for the blank (or uncatalyzed) reactions are also given in Table 1. These reactions were followed by the ^{1}H-NMR method for periods up to 2 weeks. The formation of NFM (**3c**) from formic acid and morpholine in D_2O was followed similarly in the presence of **1** (initial rate conditions) or phosphate as buffer/catalyst. Formylated intermediates such as **4** were not observed during the course of amide re-formation. The pseudo-first-order rate constants for formation of NFM in the presence of 0 and 100 mM **1** are as follows: 0.95×10^{-7} s^{-1} (200 mM in each of formic acid and morpholine, no added **1**); 1.21×10^{-7} s^{-1} (200 mM in each of formic acid and morpholine,

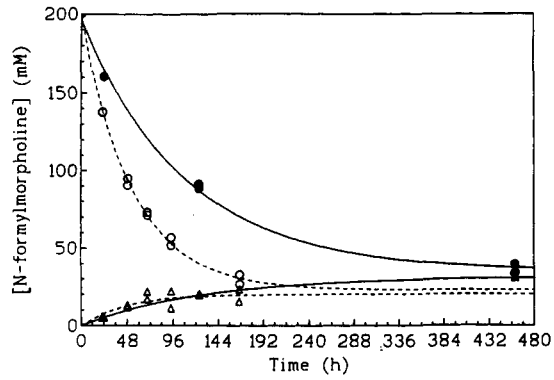


Figure 1. Establishment of equilibrium end point between NFM hydrolysis (circles) and formation (triangles) in D_2O (solid line, filled symbols) and H_2O (dashed line, open symbols): T = 98 °C, pL 7.6, μ = 1.0 (KCl), [phosphate] = 200 mM. Lines are the nonlinear least squares fits of the data and are not intended to imply rate constants. $[NFM]_{eq}$ was found to be 33 ± 3 mM in D_2O and 22 ± 3 mM in H_2O.

100 mM added **1**); 2.94×10^{-7} s^{-1} (400 mM in each of formic acid and morpholine, 100 mM added **1**).

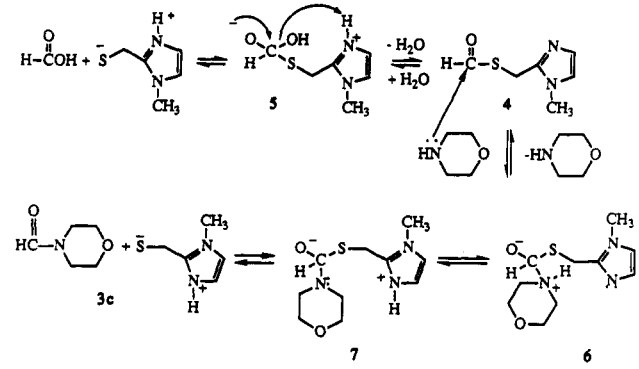
Equilibrium End Point for NFM Hydrolysis. When the reactions of NFM hydrolysis and formation were allowed to proceed to completion in D_2O as shown in Figure 1 (~6 half-times, pD 7.6, 200 mM phosphate), the equilibrium end point could be determined as 33 mM NFM, 167 mM formic acid, 167 mM morpholine. In H_2O, at pH 7.6, the reactions were ~2.5-fold faster, and the position of the end point was observed to be 22 mM NFM, 178 mM in each of formic acid and morpholine. Given the inherent errors for the ^{1}H-NMR integration and possible slight pH differences, we cannot say that the equilibrium positions in the two media are substantially different.

Partitioning of Thiolester 4. Authentic thiolester **4**, the putative intermediate in the hydrolysis of amides mediated by **1**, was synthesized and its partitioning between D_2O and amine attack at 98 °C, pD 8.0, was determined by ^{1}H-NMR. Because of the high reactivity of morpholine relative to D_2O, it was not possible to use concentrations of morpholine that were low enough to maintain both a substantial excess of the amine relative to **4**, and observe appreciable amounts of formate product. Therefore, the partitioning was determined for a less reactive amine (aniline), and subsequently the morpholine partitioning was determined by its relative reactivity compared with aniline. For aniline, $k_{aniline}/k_{hyd}$ = 71 M^{-1}, and since $k_{morph}/k_{aniline}$ = 13 ± 2, k_{morph}/k_{hyd} = 9.3×10^2 M^{-1}. Note that, in what follows, we have made the necessary assumption that the ratio of the partitioning rate constants is independent of amine concentration.

Discussion

It has been shown[4] that at 25 °C thiol imidazole **1** exists predominantly as its zwitterionic form between pH 6.5 and 9. On changing the temperature from 25 °C to 98 °C, the pK_a's of both the thiol and imidazole groups are anticipated to drop by ~0.6 units[9] ($pK_a = \Delta H°/2.303RT - \Delta S°/2.303R$). However, the increasing acidity of H_2O with temperature[10] and the change in

Scheme 1



solvent from H_2O to D_2O[11] both act to drive up the pK_a values of all weakly ionizing species in solution. Therefore, while the exact microscopic composition of **1** in terms of its zwitterionic and neutral forms at 98 °C is difficult to judge, the catalytically active[4,5] zwitterionic form ($\mathbf{1}_{ZW}$) is still anticipated to be an important component.

The mechanism by which **1** mediates the hydrolysis and reformation of amides is shown in Scheme 1 and is reasonably expected to be similar to that shown by previous work[4,5] to proceed via the involvement of an intermediate thiolester (e.g., **4**). The initial amide thiolysis part of the mechanism is analogous to that proposed by Nakken, Eldjarn, and Pihl[12] for the inactivation of penicillin using cysteine and other mercapto amines, and to our previous mechanism for acyl transfer from **2** to **1**.[5] From the latter study, the unstable intermediate **7**, formed from attack of $\mathbf{1}_{ZW}$ on **3c**, is trapped by intramolecular proton transfer from the pendant imidazolium ion to yield **6**, which subsequently expels amine to yield thiolester **4**. The hydrolysis of formyl thioester **4** is expected to be directly analogous to the hydrolysis of its acetyl derivative, which has been shown[4] to proceed by intramolecular general base assistance by the imidazole. Also emphasized in Scheme 1 is the formation of amide **3c** from its constituent acid and amine which, by microscopic reversibility, must proceed through all the same pathways and intermediates as the amide hydrolysis. That is, re-formation of thiolester **4** must proceed via attack of $\mathbf{1}_{ZW}$ on neutral formic acid followed by expulsion of the hydroxyl group with general acid catalysis from the pendant imidazolium.

During the hydrolysis of **3a–d** or re-formation of NFM, no accumulation of **4** was observed. This indicates that its breakdown (to form hydrolysis products, or products from capture by amine) must be faster than its formation. We have synthesized authentic **4** and determined that, at pH 7.6, T = 25 °C, its hydrolysis rate constant is 3.6×10^{-3} s^{-1} (0.1 M MOPS). This relatively rapid reaction would be even faster at 98 °C, which provides evidence consistent with **4** being a nonobserved intermediate.

In Scheme 2, we present a truncated form of the catalyzed pathway connecting amide **3c** with its corresponding acid and amine, and the thiolester intermediate, therein defining all the relevant equilibrium and rate constants which may be determined under the experimental conditions of T = 98 °C, pD 7.8–8.0. K'_{eq} is as defined in eq 3 and eq 4. From the phosphate equilibration

$$K'_{eq} = \frac{k_{dehyd}}{k_{hyd}}\frac{k_{form}}{k_{cat}} = \frac{k_{dehyd}}{k_{cat}}\frac{k_{form}}{k_{hyd}} = 1.2 \pm 0.2\ M^{-1} \quad (3)$$

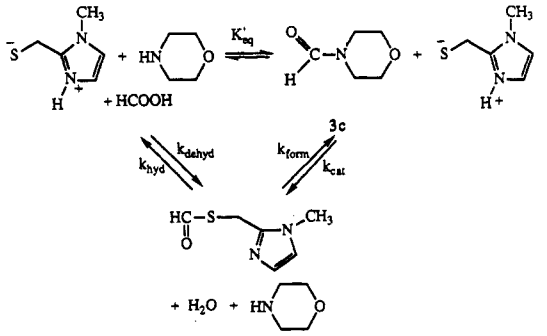
(9) (a) The $\Delta H°$ for ionization of the thiol group was estimated as being close to that of the SH in cysteine, for which a value of 11.44 kcal/mol is reported: Maoud, M. S.; Abdel-Nabby, B. A.; Soliman, E. M.; Abdel-Hamid, O. H. *Thermochim. Acta* **1988**, *128*, 75. (b) The $\Delta H°$ for ionization of the imidazolium group in **1** was estimated to be close to that for imidazolium itself, for which a value of 8.71 kcal/mol is reported: Woolley, E. M.; Wilton, R. W.; Hepler, L. G. *Can. J. Chem.* **1970**, *48*, 3249.

(10) The variation of the dissociation constant of H_2O with temperature is given as $\log K_w = -5242.39/T + 35.3944 - 0.008530T - 11.8261 \log T$ by Harned and Owen (Harned, H. S.; Owen, B. B. In *The Physical Chemistry of Electrolytic Solutions*, 3rd ed.; Reinhold Publishing Corporation: New York, 1958) (pK_w (H_2O) calcd at 98 °C = 12.3).

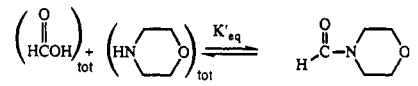
(11) The pK_w of D_2O varies with temperature in a manner similar to that of H_2O, maintaining a difference of +1 log unit in the pK_w. *The Handbook of Chemistry and Physics* (48th ed.; The Chemical Rubber Co., 1967) reports pK_w (H_2O) = 12.3 at 98 °C; therefore, pK_w (D_2O) = 13.3 at 98 °C.

(12) Nakken, K. F.; Eldjarn, L.; Pihl, A. *Biochem. Pharmacol.* **1960**, *3*, 89.

Scheme 2



experiment (Figure 1) (*vide infra*), we can experimentally define a conditional K'_{eq} (as in eq 4) as 1.2 ± 0.2 M^{-1} in D_2O at 98 °C.



(4)

While at first the observation of an equilibrium position for an amide hydrolyzing at neutral pH may be surprising, there is ample precedent (both experimental[13,14] and theoretical[15]) for this behavior. Indeed, the value for **3c** obtained here compares favorably with one indirectly determined by Fersht and Requena[13a] in H_2O, T = 25 °C (here corrected to pD 7.6) of 1.35 M^{-1}.[14]

For the catalyzed hydrolysis of **3c** under initial rate conditions of $[\text{morpholine}]_{tot} \rightarrow 0$ and steady state in [**4**], we have

$$\frac{-d[\mathbf{3c}]}{dt} = \frac{d[\text{formate}]}{dt} = \frac{k_{cat}k_{hyd}[\mathbf{3c}][\mathbf{1}]}{k_{form}[\text{morpholine}] + k_{hyd}} = k_{cat}[\mathbf{3c}][\mathbf{1}]$$

which allows determination of k_{cat} ($(1.1 \pm 0.1) \times 10^{-5}$ M^{-1} s^{-1} at 98 °C). From Scheme 2, it is seen that the propensity of intermediate **4** to form amide or hydrolyze depends upon its partitioning between morpholine and D_2O. Unfortunately, the individual rate constants, k_{form} and k_{hyd}, cannot be obtained easily at 98 °C for technical reasons (see Results). However, the partitioning ratio k_{form}/k_{hyd} can be determined easily using the integrated intensities of the formate and *N*-formyl signals generated by reaction of **4** with aqueous solutions containing known amounts of morpholine. For morpholine, the relevant value at pD 8.0, T = 98 °C, μ = 1.0 (KCl) is 9.3×10^2 M^{-1}.

Finally, we consider the term k_{dehyd} in Scheme 2, which represents the uncatalyzed rate constant for acylation of **1** by formic acid. Although we have indicated that [$\mathbf{1}_{ZW}$] does not vary appreciably with small changes in pH around neutrality, k_{dehyd} itself is pH dependent because of the linear decrease in [HCO_2H] with increasing pH above its pK_a. The conditional k_{dehyd}, unfortunately, cannot be directly determined since the pseudo-first-order rate constants for re-formation of **3c** from 200 or 400 mM each of formic acid and morpholine promoted by 100 mM **1** (1.2×10^{-7} s^{-1}; 2.9×10^{-7} s^{-1}) give evidence that the process is dependent upon both [HCO_2H] and [morpholine].[14] The term k_{dehyd} under our experimental conditions can be calculated using eq 5, a rearranged version of eq 3 (Scheme 2).

$$K_{dehyd} = K'_{eq}k_{cat}k_{hyd}/k_{form} = (1.2\ M^{-1}) \times (1.1 \times 10^{-5}\ M^{-1}\ s^{-1})(1.1 \times 10^{-3}\ M) = 1.4 \times 10^{-8}\ M^{-1}\ s^{-1} \quad (5)$$

Inspection of Scheme 2 indicates that it should be possible to determine the conditional equilibrium constant (K'_{eq}) connecting any formyl amide, and formic acid plus amine. Since k_{dehyd}, the rate constant for reaction of HCOOH with **1**, is common to the catalyzed cycle with any formyl amide, it is only necessary to determine k_{cat} and the partitioning ratio, k_{form}/k_{hyd}, as we have described above for NFM. We have applied this approach to the hydrolysis of *N*-formylaniline (formanilide), obtaining $k_{cat} = 6.5 \times 10^{-5}$ M^{-1} s^{-1} and $(k_{form}/k_{hyd})^{aniline} = 71$ M^{-1} at pD 8.0, T = 98 °C, μ = 1.0. These values, coupled to the above determined k_{dehyd}, give

$$K'_{eq} = ((1.4 \times 10^{-8}\ M^{-1}\ s^{-1})/(6.5 \times 10^{-5}\ M^{-1}\ s^{-1})) \times (71\ M^{-1}) = 0.015\ M^{-1} \quad (6)$$

This value, while determined under more severe conditions, also compares favorably with the value of 0.0052 M^{-1} (pH 8.0, T = 25 °C)[17] reported by Fersht and Requena.[13a]

The observed catalysis of the formation and hydrolysis of **3c** by phosphate buffers requires further detailed studies, but some preliminary investigations have been undertaken. Several references[19] have been made to the role of phosphate catalyzing the formation and breakdown of tetrahedral intermediates in hydrolysis reactions. The catalytic mechanisms pertinent to the present study have been suggested to be either sequential general base/general acid or bifunctional concerted general base/general acid (Scheme 3). Another, more speculative possibility exists wherein phosphate nucleophilically[20] attacks to give a transient formyl phosphate[21] intermediate. Since the general base/acid mechanisms should exhibit a solvent kinetic isotope effect (skie), the approach to equilibrium hydrolysis of NFM was repeated in H_2O, T = 98 °C, pH 7.6. The results shown in Figure 1 indicate that, on the basis of the time for the phosphate-catalyzed reaction to progress to the extent of 50% in each solvent, an approximate skie of $k_H/k_D = 2.4 \pm 0.3$ was observed, consistent with proton(s) in flight during the rate-limiting step. The end point of the reaction was observed to be slightly different in H_2O, leading to a K'_{eq} value of 0.67 ± 0.07 M^{-1}, but given the limitations of the accuracy of the ^{1}H-NMR methodology, at the 95% confidence level we cannot say that the K'_{eq} values are different.

Conclusions

Several studies have been reported wherein metal ion containing systems facilitate the hydrolysis of amides;[22] however, very little work is reported concerning a similar behavior of nonmetal systems.[12,23] In the present work, thiol imidazole **1**, designed as a crude biomimic of a cysteine protease active site, is shown to facilitate, albeit modestly, the hydrolysis of four nonactivated

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(14) The equilibrium constant defined in ref 13a takes into account the [HCOOH] and [morpholine] that would exist as neutral species at pH levels between the pK_a values of formic acid and morpholinium, e.g., $K_{eq}^{13a} = 2.24 \times 10^5$ M^{-1} = [NFM]/([$HCOO^-$H][morpholine]). The conditional equilibrium constant defined for eq 4 takes into account the total concentration of all ionized and neutral species; K'_{eq} = [NFM]/([HCOOH]$_{tot}$[morpholine]$_{tot}$) = 1.35 M^{-1}.

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(16) We thank a referee of an earlier version of this paper for pointing this out.

(17) The equilibrium constants[13a] for the equilibration of amide hydrolysis should be largely independent of pH at values between the pK_a's of the carboxylic acid and ammonium ion. For NFM, the pH independent range should extend from 4.5 to ~7.5 in H_2O, and the range should be roughly 5.0–8.0 in D_2O (pK_a (morpholinium) = 8.33; pK_a (HCO_2H) = 3.75, H_2O, T = 25 °C).[18] For formanilide, because the associated pK_a values are only 1 unit apart (pK_a (anilinium) = 4.63, H_2O, T = 25 °C),[18] the equilibrium constant decreases with increasing pH in the neutral regions.

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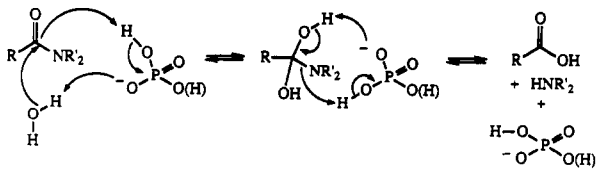
Scheme 3



formyl amides at near neutral conditions and 98 °C. The most important aspect of the present work concerns the resynthesis of amides under aqueous conditions. Although this possibility was recognized as early as 1882,[24] very little attention has been devoted to amide resynthesis under aqueous conditions.[13,15] Analysis of the mechanism of action of this catalyst provides important clues as to how this could be enhanced. Since the resynthesis route involves attack of the zwitterionic form of the catalyst on the neutral acid followed by capture by the amine, the overall process would be facilitated by adjustment of the pH or conditions such that the concentrations of all pertinent species are maximized. This could in principle be accomplished by structural modification of the catalyst so that the concentration of the zwitterionic form is maximized at pH values between the pK_a's of the carboxylic acid and ammonium ion, or by adjusting the solvent polarity such that the pK_a of the carboxylic acid is raised, thereby suppressing its ionization. It is noteworthy that the latter approach has been used for peptide resynthesis facilitated by enzymes.[3]

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Supplementary Material Available: Tables of catalyzed hydrolysis rate constants for **3a–d** as a function of **1** (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(23) A notable exception is the study of Hine et al. (Hine, J.; King, R. S.-M.; Midden, W. R.; Sinha, A. *J. Org. Chem.* **1981**, *46*, 3186–3189) in which several buffers were found to catalyze the hydrolysis of formamide.

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