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Mechanism of Hydrolysis of a Thiazolium Ion: General Acid–Base Catalysis of the Breakdown of the Tetrahedral Addition Intermediate[1]

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Rate constants in the pH range 3–9 for formation of the enethiolate product upon hydrolysis of 3,4-dimethylthiazolium ion, a model for the coenzyme thiamin, have been determined by irreversible iodination of the enethiolate at 25°C and ionic strength 1.0 M in aqueous solution. It is concluded that the rate-limiting step for hydrolysis of 3,4-dimethylthiazolium ion in the pH range 3–11 is breakdown of the neutral tetrahedral addition intermediate (T^0) to product: general acid catalysis for enethiolate formation is observed and is inconsistent with rate-limiting formation of T^0; buffer catalysis results provide no evidence for a change in rate-limiting step. Brønsted values are $\alpha = 0.53$ for general acid and $\beta = 0.31$ for general base catalysis of the formation of the hydrolysis product by oxygen-containing buffers and primary amines: nucleophilic attack of the buffer bases has been ruled out. Catalysis by buffer acids is formulated as concerted general acid catalysis of the departure of the enethiol from T^0. The buffer base- and water-catalyzed reactions are formulated as concerted general base catalysis of the expulsion of the enethiolate from T^0. It is suggested that these mechanisms are general for hydrolysis reactions of 3-substituted-4-methylthiazolium ions where the substituent on the nitrogen atom of the thiazolium ring is not an intramolecular nucleophilic catalyst.

INTRODUCTION

Thiamin (**1a**), the anti-beriberi vitamin (B_1), is composed of substituted pyrimidine and thiazolium heterocycles and is a coenzyme for a number of essential metabolic functions (*1*). Reversible hydrolysis of the thiazolium ion of thiamin in aqueous solution (Scheme 1) has received recent attention, in part because the enethiolate hydrolysis product (**2**) in the form of a mixed disulfide passes through

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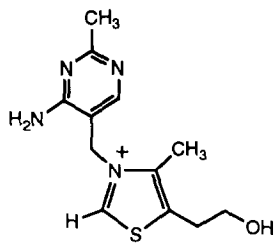
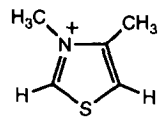
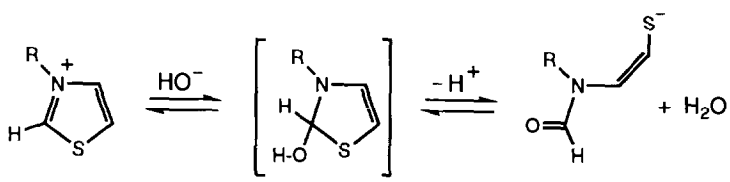


1a



1b



1 **T°** **2**

SCHEME 1

biological membranes more easily than thiamin itself and may be an obligatory intermediate in the pathway of thiamin absorption (*2*). Several key features of the hydrolysis mechanism are incompletely understood, especially in the physiological pH range; such important mechanistic considerations as the role of general acid–base catalysis and the nature of the rate-limiting steps near neutral pH have not been addressed in detail (*3*). We have reexamined the mechanism of hydrolysis of a thiazolium ion in the pH range 3–11 because of its biochemical importance and because it is a potential complicating side reaction in mechanistic investigations of thiamin-mediated catalysis.

In this paper we describe an examination of the mechanism of hydrolysis of 3,4-dimethylthiazolium ion (**1b**) in the physiological pH range. This simple thiazolium ion was selected for the initial studies because the 3-methyl substituent, unlike the 3-aminopyrimidinyl group of **1a**, is not a potential intramolecular nucleophilic catalyst so the role of the tetrahedral addition intermediate T^0 (Scheme 1) itself could be examined. Our results confirm and extend the conclusion that buffer catalysis is detectable for hydrolysis of thiazolium ions (*3*, *4*). The major revision in the hydrolysis mechanism that follows from this work is that breakdown, not formation, of T^0 is the rate-limiting step for formation of **2** in the pH range 3–11.

EXPERIMENTAL PROCEDURES

Materials. All organic chemicals were reagent grade and were purified by recrystallization or distillation. Reagent-grade inorganic chemicals were used as received. All water was prepared on a four-bowl Milli-Q water system including

an Organex-Q cartridge (Millipore). All deuterated compounds were ≥99 atom% D. The synthesis of 3,4-dimethylthiazolium iodide has been described (*5*). Stock solutions of reagents in D_2O were prepared by first exchanging exchangeable protium from the reagents with D_2O to give ≤2 atom% exchangeable protium in the stock solution except for stock iodine-triiodide solutions, which were prepared in H_2O and used at a final concentration that gave ≤4 atom% exchangeable protium in the reaction mixture. Solutions containing phosphate or amine catalysts in D_2O were prepared by partial neutralization of phosphoric acid-d_3 or the amine deuterochlorides with KOD. Solutions of the other oxygen-containing catalysts in D_2O were prepared by partial neutralization of the potassium salts with DCl.

Kinetics. Pseudo-first-order rate constants (k_{obsd}) for hydrolysis of **1b** in aqueous solution in the pH range 3–9 at 25°C and ionic strength 1.0 M, maintained with KNO_3, were obtained as previously described by spectrophotometric measurement of the zero-order disappearance of I_3^- absorption at 351 nm in solutions that contained 0.003–0.05 M **1b**, 0.10–0.15 M potassium iodide, and 4.0×10^{-5} M iodine-triiodide (*6, 7*). Reactions were initiated by addition of an aliquot from an aqueous stock solution of **1b** in H_2O or D_2O, which was prepared fresh daily and stored at 25°C. Reactions in D_2O were initiated by addition of an aliquot from an aqueous stock solution of **1b** in D_2O after C(2)-H → D exchange was complete, which eliminates the problem of the rate constants containing terms for the hydrolysis of both C(2)-H and C(2)-D thiazolium ions. Continuous absorbance changes of 0.1–0.5 were followed, the linear slope of the plot of absorbance at 351 nm against time was first order in the concentration of **1b**, and the reaction was observed to be linear until at least 90% of the iodine was consumed. We estimate error limits of ±5% in the values of k_{obsd} on the basis of the maximum and minimum slopes that could be drawn in the plots of absorbance against time assuming an error of ±0.001 in the absorbance values.

Second-order rate constants for buffer catalysis were obtained from the slopes of plots of k_{obsd} against buffer concentration (*8*). There was no curvature in the plots of k_{obsd} against buffer concentration that could be mistaken for a specific salt effect on the rate of hydrolysis (*9*). Rate constants for buffer-independent hydrolysis were obtained by extrapolation of plots of k_{obsd} to zero buffer concentration.

Rate constants in the pH range 9–11 for formation and breakdown of **2** upon hydrolysis of **1b** in aqueous solution at 25°C and ionic strength 1.0 M, maintained with KCl, were determined by following the appearance of **2** at 254 nm as described previously for **1a** (*10*). The observed first-order rate constant (k_{obsd}) for approach to equilibrium between **1b** and **2** is the sum of the rate constants for the formation (k_{HO^-}) and breakdown (k_{H^+}) reactions according to Eq. [1].

$$k_{obsd}\,(s^{-1}) = k_{HO^-}\,[HO^-] + k_{H^+}\,[H^+] \qquad [1]$$

Where duplicate determinations of k_{obsd} were made, they agreed within ±5% of the average value. Reactions with buffer components do not significantly contribute to k_{obsd} under these reaction conditions and were neglected.

Measurement of pL and calculation of the hydron and lyoxide ion concentration were performed as described elsewhere (*11*). The pK_a values of the buffer catalysts

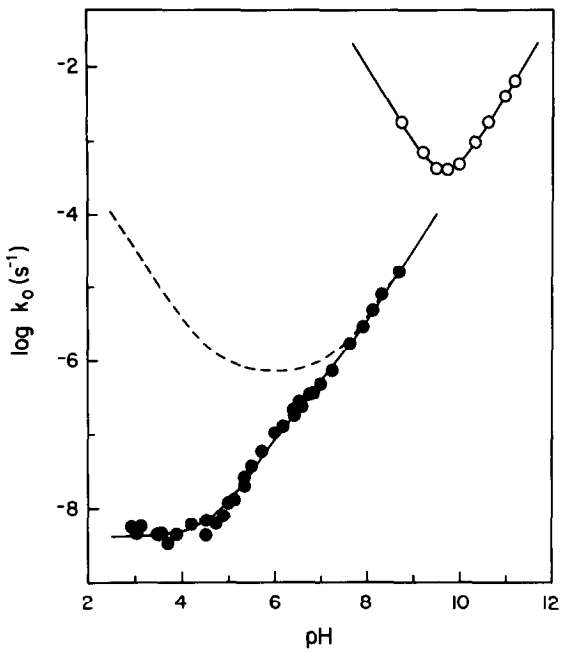


FIG. 1. Dependence on pH of the observed first-order rate constants for reversible formation in the absence of iodine (○) and irreversible formation of the enethiolate hydrolysis product (**2**) in the presence of iodine (●) from 3,4-dimethylthiazolium ion in H_2O at 25°C and ionic strength 1.0 M, maintained with KCl (○) or KNO_3 (●). Reactions with buffers do not significantly affect the pH–log rate profile above pH 9; the observed first-order rate constants were extrapolated to zero buffer concentration below pH 9. The rate constants for formation of **2** follow Eqs. [1] (○) and [3] (●), respectively, as shown by the solid lines. The dashed line describes the rate of irreversible formation of the enethiolate hydrolysis product after correction for the hydroxide ion-dependent equilibrium formation of T^0 as determined by K_{T^0} (see text).

in aqueous solution at 1.0 M ionic strength, maintained with potassium nitrate, were determined from the pL values of gravimetrically prepared buffer solutions containing the acid and base form in a 1 : 1 ratio.

RESULTS

Second-order rate constants for catalysis of enethiolate (**2**) formation from 3,4-dimethylthiazolium ion (**1b**) by added buffers in aqueous solution at 25°C and ionic strength 1.0 M (KNO_3) were determined in the pH range 3–9 by irreversibly trapping the enethiolate hydrolysis product with iodine under initial-rate conditions. Formation of **2** obeys the rate law described by Eq. [2].

$$k_{obsd}\,(s^{-1}) = k_o + k_{cat}[\text{buffer}]_{tot} \qquad [2]$$

The pseudo-first-order rate constants for buffer-independent formation of **2** (k_o) were determined as described under Experimental Procedures and are depicted in the pH–log rate profile shown in Fig. 1 (solid circles). Buffer catalysis of the

hydrolysis of **1a** and 5-(2-hydroxyethyl)-3,4-dimethylthiazolium ion has been previously reported (*3, 4*).

To our knowledge a halogenation reaction has not previously been used in a *continuous* assay to follow the ring opening of a thiazolium ion. The determination of thiols by direct titration of the thiolate with triiodide ion (*12a*) and the irreversible iodination of **2** in basic aqueous solution in a *discontinuous* assay have been described (*12b,c*). The following evidence supports the previous conclusion (*12b,c*) that the iodine trapping assay is following the rate of hydrolysis of a thiazolium ion:

(i) The iodine assay gives identical values of k_{obsd} in the pH range 4–8 as a disulfide interchange assay[3] and a polarographic assay (*13*). Previous workers demonstrated that rates of disulfide interchange (*4*) and the heights of anodic waves (*13*) are proportional to the concentration of enethiolate (**2**). This establishes that the iodination reaction is following the formation of **2** in the pH range 3–9, that the trapping products are the same in the pH region 4–8, and that the stoichiometry of the trapping reaction involves one molecule of iodine (*14*). The identical values of k_{obsd} obtained with the iodine, disulfide interchange, and polarographic assays also indicate that iodination of the C(2)-ylide, if it occurs, is readily reversible. Very different values of k_{obsd} would have been observed if a nearly diffusion-controlled reaction between the C(2)-ylide and iodine were kinetically significant because catalysis by hydroxide ion of C(2)-H exchange to form the C(2)-ylide is 8×10^4-fold faster than formation of **2** (*11*).

(ii) The reaction rate displayed a first-order dependence on the concentration of substrate and was independent of the concentration of the iodine trap. This means that the rate of trapping is always faster than the rate of ring opening.

(iii) The iodination reaction is not readily reversible because iodine was completely consumed for iodination of **1b** in the pH range 3–9 and there was no significant increase in absorbance at 351 nm after prolonged incubation of the iodinated substrate at pH 3.5 that would indicate the loss of I^+ (*15*).

An advantage of irreversibly trapping **2** for mechanistic investigations at pH $\leq$ 8, where ring opening proceeds to a small extent, over assays that follow ring closure of **2** to form the thiazolium ion is that determination of the relatively slow observed rate of appearance of **2** by iodine trapping does not require rapid kinetic methods (*3, 4*).

Figure 1 also shows the dependence on pH of the observed pseudo-first-order rate constants for reversible formation of **2** in the absence of iodine in the pH range 9–11 (open circles). The pH–log rate profile for hydrolysis of **1b** is consistent with the approach to an equilibrium concentration of **2** in the pH range 9–11 (*10*). Values of $k_{HO^-} = 2.3\ M^{-1}\ s^{-1}$ and $k_{H^+} = 1.1 \times 10^6\ M^{-1}\ s^{-1}$, calculated from Eq. [1], agree reasonably well with literature values for k_{HO^-} [$3\ M^{-1}\ s^{-1}$ (30°C) (*16*), 4

[3] Values for k_{obsd} were determined in the pH range 4–8 using the iodine trapping assay or by trapping **2** by a disulfide interchange reaction with 5,5-dithiobis(2-nitrobenzoate) ($DTNB^{2-}$) and following the release of the chromophoric product, 2-nitro-5-thiobenzoate (TNB^{2-}) (*4*). Identical values of k_{obsd} were obtained if the disulfide interchange reaction was performed in deoxygenated buffers under a nitrogen atmosphere to prevent oxidation of TNB^{2-} to $DTNB^{2-}$ (*36*).

M^{-1} s^{-1} (25°C) (*13*), 8.6 M^{-1} s^{-1} (20°C, I = 0.1 M) (*17*), 11.5 M^{-1} s^{-2} (30°C, I = 0.1 M) (*17*)] and k_{H^+} [1 × 10^7 M^{-1} s^{-1} (25°C) (*13*), 8.3 × 10^5 M^{-1} s^{-1} (30°C, I = 0.1 M) (*17*)] obtained with this and other assays for **2**.

The pseudo-first-order rate constants shown in the pH–log rate profile in Fig. 1 for irreversible formation of **2** determined in the pH range 3–9 (solid circles) obey the rate law outlined in Eq. [3].

$$k_o\,(s^{-1}) = (k_{H_2O} + k_{H^+}[H^+] + k_{HO^-}[OH^-])/(1 + [H^+]/K_W K_{T^0}) \qquad [3]$$

The dashed line in Fig. 1 represents the curve determined from the rate constants for proton-, water-, and hydroxide ion-catalyzed formation of **2**, corrected for a hydroxide ion-dependent equilibrium represented by K_{T^0}. Values of $k_{H_2O}/[H_2O]$ = 7.4 × 10^{-7} s^{-1}/55.4 M = 1.3 × 10^{-8} M^{-1} s^{-1}, k_{H^+} = 3.0 × 10^{-2} M^{-1} s^{-1}, and k_{HO^-} = 2.3 M^{-1} s^{-1} for formation of **2** were calculated using Eq. [3] with K_W = $10^{-14.000}$ (*18*) and K_{T^0} = $10^{6.9}$ M^{-1}. The ratio k_{HO^-}/k_{H_2O} = 3 × 10^6 M^{-1} is similar to the ratio of 10^7 M^{-1} observed for several other heterocyclic iminium ions (*19*). This pH–log rate profile for *irreversible* formation of **2** derived from **1b** (Fig. 1) is not directly comparable to the previously reported pH–log rate profile for *reversible* reclosure of the enethiolate derived from 5-(2-hydroxyethyl)-3,4-dimethylthiazolium ion in the pH range 1–10 (*3*).

The pH–log rate profile for the reaction of **1b** in the pH range 3–9 (Fig. 1, solid line) indicates a change in the pathway for enethiolate formation by the upward deviation in the profile at pH ≈ 4 from pH-independent H_2O to hydroxide ion-catalyzed enethiolate formation. There is a downward deviation in the profile near pH 6.9 to a second pH-independent region followed by another hydroxide ion-catalyzed region at pH > 8: the rate constants for hydroxide ion-catalyzed enethiolate formation in the region pH 5–6 and pH > 8 are twofold different and cannot be accounted for by a single value of k_{HO^-} through the pH range 5–9. The downward deviation near pH 6.9 (Fig. 1) may represent either a change in rate-limiting step or a pH-dependent equilibrium reaction that occurs to the reactant **1b** prior to the rate-limiting step for ring-opening (*8*).

The observed buffer catalysis for formation of **2** obeys the rate law outlined in Eq. [4].

$$(k_{obsd} - k_o)(1 + [H^+]/K_W K_{T^0}) = k'_{cat}[\text{buffer}]_{tot} = k_{BH^+}[BH^+] + k_B[B] \qquad [4]$$

Figure 2 shows a plot of k_{obsd}, corrected for the hydroxide ion-dependent equilibrium representing K_{T^0} (see Scheme 2 and Eq. [5] below), against total acetate buffer concentration. The values of the second-order rate constants for the general base- and general acid-catalyzed formation of **2** were obtained from plots of the second-order rate constants for buffer catalysis, k'_{cat}, against percentage buffer base and extrapolating to 100 and 0% free base, respectively. Values of the second-order rate constants for general base and general acid catalysis are summarized in Tables 1 and 2, respectively. Experiments with other buffer catalysts were carried out in the same manner as those shown for acetate ion with the same number of observed rate constants.

Solvent deuterium isotope effects on the formation of **2** catalyzed by solvent

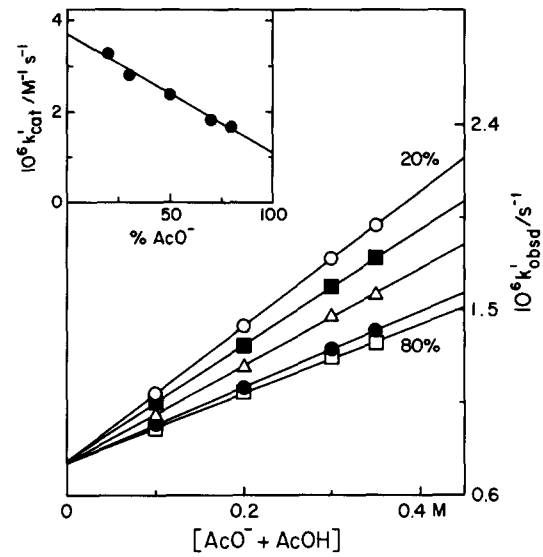


FIG. 2. Dependence of the observed rate constants for irreversible formation of the enethiolate hydrolysis product from 3,4-dimethylthiazolium ion in the presence of iodine on the concentration of acetate buffer at 25°C and I = 1.0 M (KNO_3) in H_2O. Values of k_{obsd} are corrected for the hydroxide ion-dependent equilibrium formation of T^0 as determined by K_{T^0} (see text). The buffers were 20% (○), 30% (■), 50% (△), 70% (●), and 80% (□) free base; the slope of the plot against buffer concentration gives the apparent rate constant of the acetate buffer-catalyzed reaction, k'_{cat} (see text). The inset shows the dependence of the apparent catalytic constant for acetate buffer catalysis, k'_{cat}, on the composition of the acetate buffer.

(D_2O), deuteroxide ion, buffer bases, and buffer acids were determined using the iodine trapping assay under initial rate conditions at 25°C, I = 1.0 M (KNO_3). The rate constant for lyoxide ion-catalyzed formation of **2** was determined from the slope of a plot of observed rate constants, extrapolated to zero buffer concentration, against lyoxide ion concentration. The rate constant of the pL-independent (L = H or D) "water" reaction was obtained from the intercepts of these plots at zero lyoxide ion concentration. Values of $k_{D_2O}/(D_2O] = 1.8 \times 10^{-7}$ $s^{-1}/55.1$ M = 3.3×10^{-9} M^{-1} s^{-1}, and $k_{DO^-} = 5.5$ M^{-1} s^{-1} were obtained, which give solvent isotope effects of $k_{H_2O}/k_{D_2O} = 4.1$ and $k_{DO^-}/k_{HO^-} = 2.4$ for formation of **2**.

The values of $(k_{H_2O}/k_{D_2O})_B$ for catalysis of the formation of **2** by oxygen-containing buffer bases and primary amines are in the range 1.6–4.7, exhibit a maximum in the pH-independent region of the pH–log rate profile (Fig. 1, dashed line), and are summarized in Table 1. The values of $(k_{H_2O}/k_{D_2O})_{BL^+}$ for catalysis by buffer acids increase in the range 1.0–4.1 with increasing buffer acidity and are summarized in Table 2. Values of k_B for catalysis by buffer bases in L_2O and k_{BL^+} for catalysis by buffer acids in L_2O are reported in Tables 1 and 2, respectively. The uncertainty in the isotope effects is ± 0.2 on the basis of the errors in the observed rate constants.

TABLE 1

General Base Catalysis of 3,4-Dimethylthiazolium Ion Hydrolysis[a]

Catalyst	pK_a[b]	L_2O	k_B, $M^{-1}\ s^{-1}$	$(k_{H_2O}/k_{D_2O})_B$
L_2O[c]	−1.74	H_2O	1.3×10^{-8}	3.9
		D_2O	3.3×10^{-9}	
$NCCH_2COO^-$	2.35	H_2O	3.0×10^{-7}	1.6
		D_2O	1.9×10^{-7}	
$ClCH_2COO^-$	2.65	H_2O	3.7×10^{-7}	
$CH_3OCH_2COO^-$	3.49	H_2O	7.6×10^{-7}	
CH_3COO^-	4.64	H_2O	1.1×10^{-6}	2.3
		D_2O	4.9×10^{-7}	
$CF_3CH_2NL_2$	5.73	H_2O	7.2×10^{-6}	3.1
		D_2O	2.3×10^{-6}	
$(CH_3)_2AsO_2^-$	6.20	H_2O	2.2×10^{-6}	
LPO_4^{2-}	6.49	H_2O	7.1×10^{-6}	4.7
		D_2O	1.5×10^{-6}	
$CH_3CH_2PO_3^{2-}$	7.60	H_2O	1.6×10^{-5}	
$(LOCH_2)_3CNL_2$	8.31	H_2O	1.9×10^{-5}	1.7
		D_2O	1.1×10^{-5}	

[a] At 25°C and ionic strength 1.0 M (KNO_3) in L_2O. The rate constant k_B is defined in Eq. [4].
[b] Apparent pK_a of the conjugate acid at 25°C and ionic strength 1.0 M (KNO_3) in H_2O (see text).
[c] Second-order rate constants were calculated from the observed pseudo-first-order rate constants on the basis of a standard state of 55.4 M for pure H_2O and 55.1 M for pure D_2O at 25°C.

TABLE 2

General Acid Catalysis of 3,4-Dimethylthiazolium Ion Hydrolysis[a]

Catalyst	pK_a[b]	L_2O	k_{BL^+}, $M^{-1}\ s^{-1}$	$(k_{H_2O}/k_{D_2O})_{BL^+}$
H_3O^+	−1.74	H_2O	3.0×10^{-2}	
$NCCH_2COOL$	2.35	H_2O	1.6×10^{-4}	4.1
		D_2O	3.9×10^{-5}	
$ClCH_2COOH$	2.65	H_2O	1.1×10^{-4}	
CH_3OCH_2COOH	3.49	H_2O	5.3×10^{-5}	
CH_3COOL	4.64	H_2O	3.7×10^{-6}	2.5
		D_2O	1.5×10^{-6}	
$CF_3CH_2NL_3^+$	5.73	H_2O	1.1×10^{-5}	1.5
		D_2O	7.4×10^{-6}	
$(CH_3)_2AsO_2H$	6.20	H_2O	2.7×10^{-6}	
$L_2PO_4^-$	6.49	H_2O	3.6×10^{-7}	≥ 1.0
		D_2O	$\leq 3.6 \times 10^{-7}$	
$CH_3CH_2PO_3H^-$	7.60	H_2O	3.3×10^{-7}	
$(HOCH_2)_3CNH_3^+$	8.31	H_2O	$\leq 8.9 \times 10^{-8}$	

[a] At 25°C and ionic strength 1.0 M (KNO_3) in L_2O. The rate constant k_{BL^+} is defined in Eq. [4].
[b] Apparent pK_a at 25°C and ionic strength 1.0 M (KNO_3) in H_2O (see text).

DISCUSSION

Mechanistic investigations of nonenzymatic reactions involving thiamin (**1a**) and 2-substituted-thiamin analogs in aqueous solution can be complicated by hydrolysis of the thiazolium ring (*10*). A goal of this work was to examine the hydrolysis reaction of 3,4-dimethylthiazolium ion (**1b**) in the physiological pH range to establish the pH–log rate profile, the efficiency of catalysis by buffer acids and bases, and the nature of the rate-limiting steps for comparison with other reactions in this pH range.

The nature of the rate-limiting step. Several groups have evaluated the rate of hydrolysis of simple 3-alkylthiazolium ions in basic aqueous solution and concluded that the formation of T^0 is rate-limiting at pH $\geq$ 9 (*20*). The fact that the rate constant of 2.3 $M^{-1} s^{-1}$ for hydroxide ion catalysis of the irreversible formation of **2** in the pH range 7–9 is identical to the value for hydroxide ion-catalyzed formation of **2** at pH $\geq$ 10 would suggest that formation of **2** involves rate-limiting formation of T^0 at pH $\geq$ 7. This conclusion can be ruled out, however, based on the results described in this work.

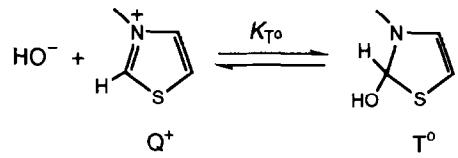
Although the downward deviation (nonlinearity) exhibited in Fig. 1 near pH 6.9 would suggest a change in rate-limiting step from rate-limiting formation of T^0 at high pH to rate-limiting breakdown of T^0 at low pH, the lack of evidence for a change in rate-limiting step in plots of the rate constants for formation of **2** against buffer concentration at or near pH 6.9 is inconsistent with the downward deviation in Fig. 1 representing a change in rate-limiting step for formation of **2**. The plots for catalysis by phosphate, ethylphosphonate, and tris(hydroxymethyl)aminomethane buffers resemble the plots shown in Fig. 2 for catalysis by acetate buffers and are linear in the pH range 6–8, rather than curved. Furthermore, plots of the second-order rate constants for buffer catalysis (k_{cat}, Eq. [2]) against percentage buffer base are not linear (results not shown): this suggests that there is a pH-dependent equilibrium reaction (Scheme 2, below) that occurs to the reactant **1b** prior to the rate-limiting step for ring-opening (*8*).

Since there is no evidence for a change in rate-limiting step for formation of **2** in the pH range 3–9, the pathways for hydrolysis shown in Fig. 1 must therefore represent either rate-limiting formation, or rate-limiting breakdown, of T^0. Because observed general acid catalysis of the formation of **2** cannot be reasonably assigned to a step involving formation of T^0, the hydrolysis of 3,4-dimethylthiazolium ion must therefore involve rate-limiting breakdown of the tetrahedral addition intermediate T^0 in the pH range 3–9.

The conclusion that breakdown of T^0 is rate-limiting in the pH range 3–9 suggests that the downward deviation (nonlinearity) observed near pH 6.9 represents the pH-dependent addition of hydroxide ion at equilibrium to free thiazolium ion prior to ring-opening. This pH-dependent equilibrium is described by Scheme 2 and the equilibrium constant for T^0 (pseudobase) formation given in Eq. [5].

$$K_{R^+} = K_W K_{T^0} = [H^+][T^0]/[Q^+] \qquad [5]$$

The equilibrium constant K_{R^+} is analogous to the pK_a of a Brønsted acid and is the hydrogen ion concentration at which T^0 and the thiazolium ion (Q^+) are present



SCHEME 2

in equal amounts. The apparent pK_a value of 6.9 that we attribute to pseudobase formation in **1b** is similar to the pK_{R^+} value of 7.15 for another nitrogen heterocycle, 1,3-dimethyl-2-oxopyrimidinium ion (*19b*).[4] The dashed line in Fig. 1, which describes the rate of irreversible formation of the enethiolate hydrolysis product after correction of the values of k_{obsd} (extrapolated to zero buffer concentration) for the hydroxide ion-dependent equilibrium representing K_{T^0} according to Eq. [3], resembles the profile obtained for breakdown of the water addition intermediate derived from another iminium ion, phthalimidium ion, except that the upward deviations occur at lower pH values (*21*).

The hydroxide ion-catalyzed reaction at pH > 8 must proceed by a mechanism involving specific-base catalysis, in which the intermediate anion T^- is formed from T^0 at equilibrium followed by rate-limiting breakdown of T^- to product.[5] Formation of T^- through rate-limiting proton transfer can be excluded as a mechanism because the Brønsted plot for general base catalysis (Fig. 3) is inconsistent with an "Eigen curve" typical for general acid–base-catalyzed reactions involving rate-limiting proton transfers (*22*): thermodynamically unfavorable rate-limiting proton transfer from T^0 to water and buffer bases would give a Brønsted slope of $\beta = 1.0$ rather than the observed slope of $\beta = 0.31 \pm 0.03$ for general base catalysis of the formation of **2**. The large value of the secondary solvent isotope effect of $k_{DO^-}/k_{HO^-} = 2.4$ is consistent with a mechanism involving an anionic species in a preequilibrium step and little or no kinetic isotope effect on the rate constant for unassisted breakdown of T^- (*24*).

General-base catalyzed breakdown of T^0 by hydroxide ion is unlikely because proton removal from T^0 by hydroxide ion is thermodynamically favorable (*25*), and, consequently, provides no driving force for concerted catalysis (*26*). The absence of significant buffer catalysis at pH ≥ 8 and the fact that the rate constant for hydroxide ion-catalyzed formation of **2** is some 900-fold faster than that predicted from the Brønsted plot for general base catalysis (Fig. 3) are in accord with

[4] No changes in the ultraviolet/visible spectrum of **1b** that might suggest the presence of T^0 were detected in the pH range 3–9. However, there is a pH-dependent upfield shift (≈2 ppm) with an apparent pK_a value of ≈7 of the resonances assigned to the N(3)–CH_3 (43 ppm) and N(3)–CH_2-groups (49 ppm) in the noise-decoupled ^{13}C NMR spectrum of **1b** and oxythiamin, respectively (data not shown), which is consistent with the presence of T^0 at equilibrium (*20*). The ^{13}C NMR spectra were recorded at 27°C in 42 mM potassium phosphate buffer (pH 6–8) in 15% D_2O at $I = 1.0$ M (KCl).

[5] A value of $pK_a = 13 \pm 1$ for T^0 was estimated from a Hammett correlation for the ionization of secondary alcohols, which follow $pK_a^{R'R''CH(OH)} = 15.9 - 1.42\Sigma\sigma^*$ (*37*), and $\sigma^* = 0.32$ for R′ = $-N(CH_3)_2$ and $\sigma^* = 1.31$ for R″ = $-SCH{=}CH_2$ (*38*).

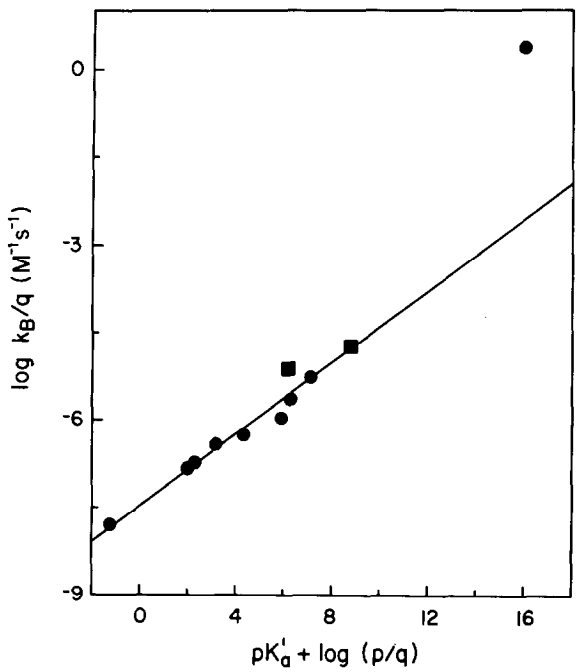


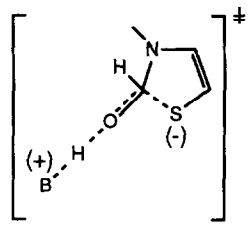
FIG. 3. Brønsted plot for general base catalysis of the irreversible formation of the enethiolate hydrolysis product during hydrolysis of 3,4-dimethylthiazolium ion in the presence of iodine in the pH range 3–9 at 25°C and I = 1.0 M (KNO_3) in H_2O. Statistical corrections were made according to Bell and Evans (*23*). The line of slope 0.31 ± 0.03 is the best fit through the rate constants for oxygen-containing buffers (circles) and primary amines (squares).

this formulation. This breakdown reaction does not involve general acid catalysis by water because there is little or no thermodynamic advantage to proton transfer from water to the leaving enethiolate ion (*26*).

The observed Brønsted slope of β = 0.31 (Fig. 3) for the observed general-base-catalyzed formation of **2** in the pH range 5–8 is consistent with catalysis involving a concerted mechanism (*21*).[6,7] The proposed mechanism, **3**, represents a nonen-

[6] This minimal explanation of the data is consistent with the pH–log rate profile (Fig. 1) and a single Brønsted line defined by this heterogeneous set of buffers (Figs. 3 and 4). The ≤fourfold deviations of the second-order rate constants for general base and general acid catalysis by the buffers are not unusual, are randomly scattered about the Brønsted line, and provide no evidence that a unique Brønsted correlation is required for each buffer class.

[7] Nucleophilic catalysis can be ruled out for two reasons. First, the reaction shows little sensitivity to steric requirements as is shown, for example, by the reactivity of 2,2,2-trifluoroethylamine and tris(hydroxymethyl)aminomethane. Nucleophilic reactions of carbonyl compounds show marked sensitivity to steric hindrance in the nucleophile but general acid–base catalysis is much less sensitive to steric effects. Second, the catalytic constants of the bases for **1b** do not deviate more than a factor of three from the line in Fig. 3 (with the exception of HO^-). In contrast, the rate constants for the reactions of nucleophilic reagents of similar basicity but different structure with carbonyl compounds vary over a range of several orders of magnitude [see, for example, (*39*)].



3

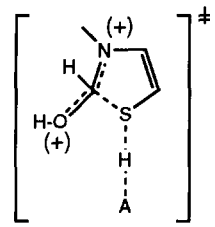
forced class *e* mechanism for the breakdown of T^0, in which a proton is removed from the oxygen atom by the general base; in the reverse direction it involves general acid catalysis of RS^- attack.[8] This is consistent with the previous suggestion that addition of a relatively weak nucleophile, such as RSH, to a carbonyl group would involve a fully concerted class *e* mechanism of general acid catalysis, in which the catalyst interacts with the electrophilic carbonyl group (*27*) and the proposed class *e* mechanism for catalysis of the hydrolysis of **1a** by glycine buffers (*4*). According to mechanism **3**, the pH-independent water reaction represents general base catalysis by water of the breakdown of T^0. The rate constant for catalysis by water falls on the extrapolated Brønsted line for general base catalysis, in accord with this formulation.

There is a change to a third pathway for breakdown of T^0 to product below pH 5. Evidence for a third pathway for breakdown of T^0 includes: (i) the upward deviation in the pH–log rate profile (Fig. 1, dashed line) at pH 5 from pH-independent water to H^+-catalyzed hydrolysis; (ii) the increasing contribution of general acid catalysis of the formation of **2** with decreasing pH, which gives a Brønsted plot of slope $\alpha = 0.53 \pm 0.06$ (Fig. 4); and (iii) an increase in the primary kinetic isotope effect on the observed general acid catalysis of $(k_{H_2O}/k_{D_2O})_{BL^+}$ from 1.0 to 4.1 (Table 2) with decreasing pH.

The mechanism of the general acid-catalyzed reaction below pH 5 is assigned to general-acid catalysis of the breakdown of the neutral intermediate T^0 rather than to the kinetically equivalent specific acid–general base catalysis of the breakdown of a cationic intermediate involving an extremely unstable S-protonated hydrate. The observed general acid catalysis is consistent with a concerted mechanism of catalysis of the expulsion of the leaving enethiolate from T^0 by proton donation (**4**), which is similar to the proposed mechanism of general acid-catalyzed breakdown of T^0 derived from a phthalimidium ion (*21*). To our knowledge this is the first demonstration of catalysis of this kind at sulfur (*8*).

It is likely that these mechanisms are general for hydrolysis reactions of simple 3-R-4-methylthiazolium ions where the substituent on the nitrogen atom of the

[8] A value of $pK_a \approx 9$ for **2** was estimated from a Hammett correlation for the ionization of thiols, which follow $pK_a^{RSH} = 10.22 - 3.50\sigma^*$ (*40*), and $\sigma^* = 0.36$ for R = $-CH{=}CHCH_3$ (*41*). A pK_a value of 7.9 was determined from the pH–log rate profile for reclosure of the enethiolate derived from 5-(2-hydroxyethyl)-3,4-dimethylthiazolium ion (*3*).



4

thiazolium ring is not a potential intramolecular nucleophilic catalyst because a similar pH–log rate profile and pattern of general acid–base catalysis was obtained for irreversible hydrolysis of 3-benzyl-4-methylthiazolium ion, *N*(1′)-methylthiamin, and oxythiamin (*28*). N(1′)-unsubstituted ("free") thiamin (**1a**) is not a simple thiazolium ion in this respect because the exocyclic 4′-amino group is an intramolecular nucleophilic catalyst of the ring-opening reaction in neutral aqueous solu-

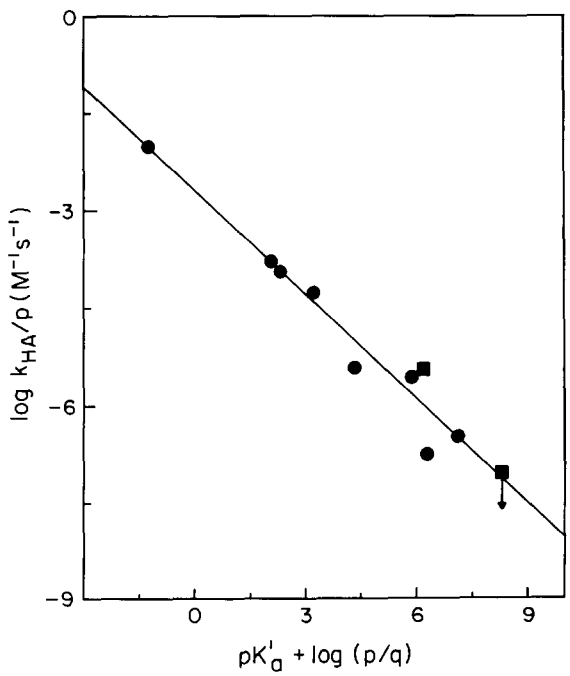


FIG. 4. Brønsted plot for general acid catalysis of the irreversible formation of the enethiolate hydrolysis product during hydrolysis of 3,4-dimethylthiazolium ion in the presence of iodine in the pH range 3–9 at 25°C and I = 1.0 M (KNO_3) in H_2O. Statistical corrections were made according to Bell and Evans (*23*). The upper limit for catalysis by protonated tris(hydroxymethyl)aminomethane is indicated. The line of slope 0.53 ± 0.06 is the best fit through the oxygen-containing buffers (circles) and primary amines (squares).

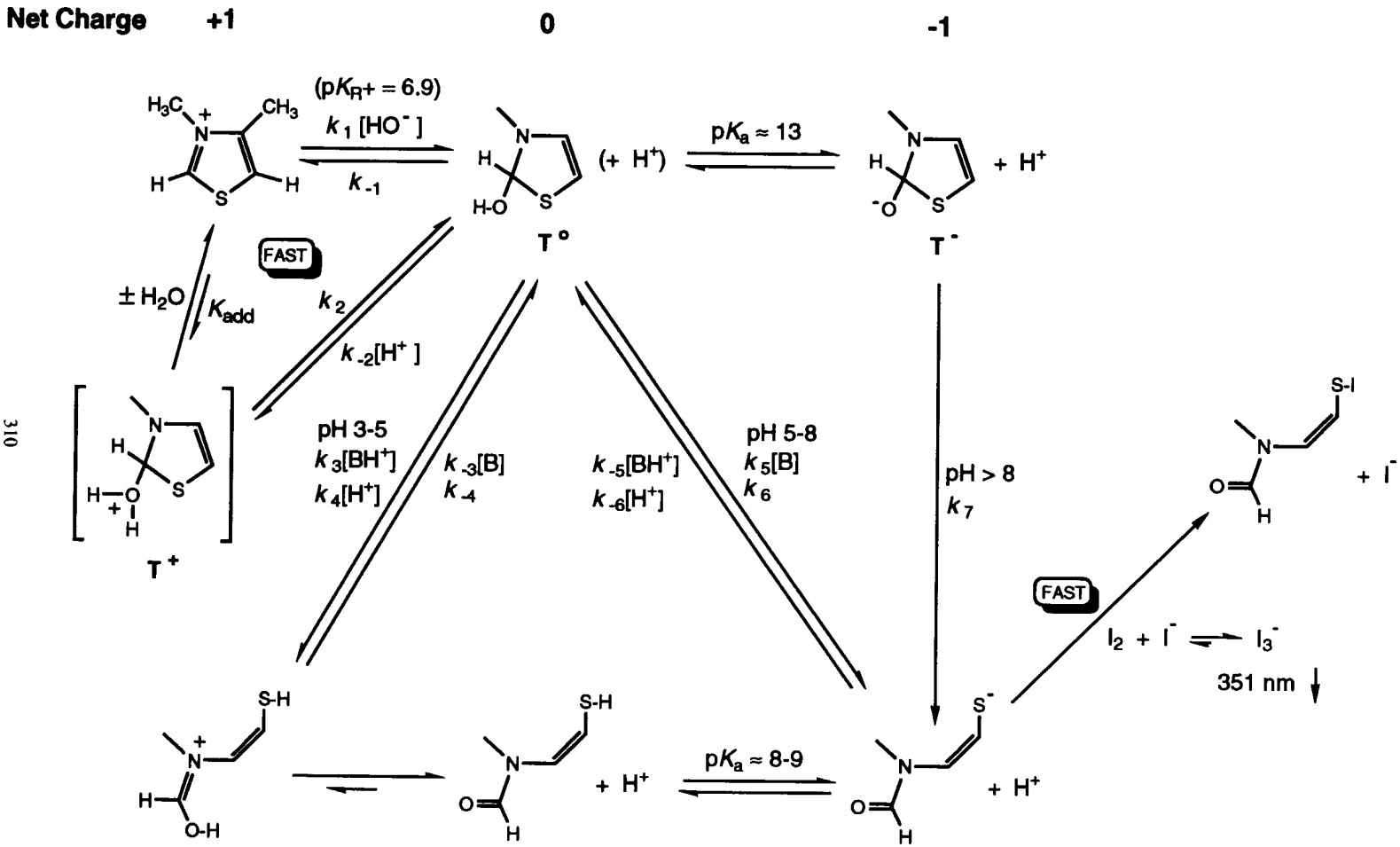
tion (*28*). The 4′-amino group also participates in an intramolecular cyclization with the thiazolium ring in basic solution to form a neutral tricyclic species (*29*) and adds to the acetyl group of 2-acetyl-TDP in neutral aqueous solution (*30*). The ring-opening mechanism of free thiamin and the stability of intramolecular amine addition compounds formed from thiazolium ions will be examined further in a following paper (*28*).

The isotope effect maximum. The maximum in the values of $(k_{H_2O}/k_{D_2O})_B$ (Table 1) for catalysis by oxygen-containing buffer bases and primary amines in the pH range 3–8 can be accounted for by development of asymmetry in the transition state for hydron transfer because a constant kinetic isotope effect is expected for the concerted reaction mechanism (**3**).[9] The maxima for general acid catalysis of the addition of methoxyamine to phenyl acetate (*31*) and general base catalysis by monofunctional nitrogen bases of the addition of aniline to methyl formate (*32*) are consistent with a changing isotope effect on the proton transfer step itself; this is caused by a sharp change from a symmetric to an asymmetric transition state for this very rapid proton transfer step (*33*). The Brønsted plot for general base catalysis of the breakdown of T^0 (Fig. 3) is linear, rather than curved, which is inconsistent with predominantly rate-limiting diffusional steps accounting for the solvent isotope effect maximum (*34*). The pH–log rate profile for enethiolate formation in the pH range 3–9 (Fig. 1) is also inconsistent with changes in the rate-limiting step for enethiolate formation.

Summary. Three conclusions that follow from this work and represent significant revisions in the mechanism for hydrolysis of a simple 3-alkylthiazolium ion are (i) that the rate-limiting step for hydrolysis of 3,4-dimethylthiazolium ion (**1b**) in the pH range 3–11 is breakdown of the neutral tetrahedral addition intermediate (T^0) to product; (ii) that there are three pathways for breakdown of T^0 to product in the pH range 3–11; and (iii) that the key factor in determining which of the three pathways for breakdown of T^0 is followed is the ability of this intermediate to lose a proton from its hydroxyl group.

A mechanism for the hydrolysis of a simple 3-alkylthiazolium ion that is consistent with the results is outlined in Scheme 3. This mechanism is a refinement of the mechanism outlined in Scheme 1 for the hydrolysis of a 3-alkylthiazolium ion in basic aqueous solution because it includes steps involving general acid–base catalysis of the formation and breakdown of tetrahedral addition intermediates which become important in the physiological pH range and have not been addressed in previous mechanistic studies on thiazolium ions. The mechanism for hydrolysis of other iminium ions (*19*) also proceeds in a stepwise manner through tetrahedral addition intermediates involving general acid–base catalysis of the formation and breakdown of these tetrahedral intermediates (*21, 35*).

[9] A small ($k_H/k_D \leq 1.4$) contribution from a secondary α-deuterium kinetic isotope effect for conversion of C(2) from sp^3 to sp^2 hybridization in rate-limiting breakdown of T^0 is unavoidable in these experiments (*42*). Thiazolium C(2)–H exchange is so much faster than hydrolysis of the thiazolium ion (*11*) that it is not possible to have the majority of the substrate in the C(2)–H form and observe a significant extent of the hydrolysis reaction in D_2O.


Net Charge
+1
0
-1
(pK_R+ = 6.9)
k_1 [HO^-]
k_-1
pK_a ≈ 13
(+ H^+)
+ H^+
T°
T^-
T^+
FAST
± H_2O
K_add
k_2
k_-2[H^+]
pH 3-5
k_3[BH^+]
k_4[H^+]
k_-3[B]
k_-4
k_-5[BH^+]
k_-6[H^+]
pH 5-8
k_5[B]
k_6
pH > 8
k_7
FAST
I_2 + I^- ⇌ I_3^-
351 nm
+ I^-
pK_a ≈ 8-9
+ H^+
+ H^+

SCHEME 3

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