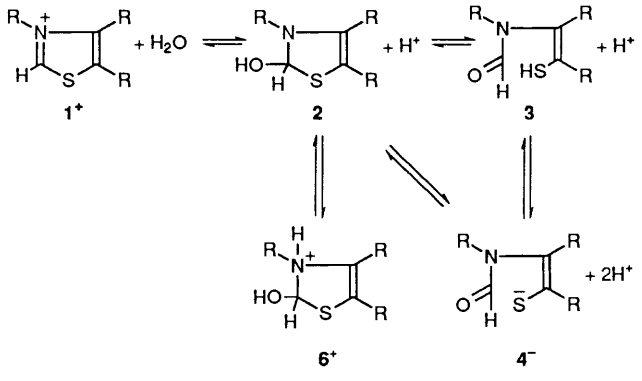
Kinetic Study on the Hydrolysis of Thiazolium Cations

Susanne Barrabass, Irmgard Heiber-Langer and Wilhelm Knoche
Fakultät für Chemie, Universität Bielefeld, D-33501 Bielefeld, Germany

Using the stopped-flow technique the hydrolysis of ten differently substituted thiazolium cations has been studied. For all compounds the hydrolysis follows the same reaction scheme leading to a ring-opened anion. A pseudo base, the protonated pseudo base and the protonated ring-opened anion exist as intermediates. Rate and equilibrium constants were determined for the different steps involved in the reaction.

In a previous communication[1] we have reported on a kinetic study concerning the hydrolysis of the 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium cation. The results led to Scheme 1 for this reaction, where at equilibrium only species $\mathbf{1}^+$ and $\mathbf{4}^-$ exist in significant concentrations. Species **2**, **3**, and $\mathbf{6}^+$ could be detected as intermediates, when acid was added to basic solutions containing species $\mathbf{4}^-$. According to Breslow[2] an ylide is the catalytically active form of thiazolium compounds, which, however, in the range pH > 15 could not be observed, in agreement with results reported by Washabaugh and Jencks[3] for different thiazolium salts. (For results obtained by other authors, please see the literature quoted in ref. 1.) All equilibrium constants involved in reaction Scheme 1 were determined. Applying the stopped-flow technique we could follow the reaction steps $\mathbf{1}^+ \rightleftharpoons \mathbf{2}$ and $\mathbf{2} \rightleftharpoons \mathbf{3}$, whereas the speed of the other steps is too high to be measured.



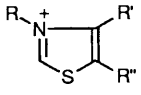
Scheme 1

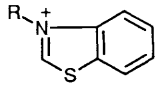
The most important representative of the thiazoles is thiamine pyrophosphate (vitamin B_1), where substituent R comprises a pyrimidine moiety. The pyrimidine ring undergoes a further protonation reaction and therefore the hydrolysis of this compound is more complicated than indicated by Scheme 1. Therefore before studying the hydrolysis of thiamine, in this contribution we report on the protonation of differently substituted thiazolium cations. Table 1 shows the substituents and the abbreviations used for the different compounds; for EaB two substituents are replaced by a benzene annelated to the thiazole ring.

Experimental

The thiazolium salts were prepared from 5-(2-hydroxyethyl)-4-methyl-1,3-thiazole or 4-methyl-1,3-thiazole and suitable benzyl halides or methyl iodide as described in the literature.[4,5] All other chemicals used are commercially available and of analytical grade. The solutions were prepared with triply distilled water. The aqueous stock solutions of thiazolium salts were prepared weekly; the reactant mixtures were prepared immediately before the measurements. Buffer solutions were prepared according to the data collected in Robinson/Stokes.[6] Activity corrections were calculated from the ionic strength using Davies equation.[7]

Table 1 Abbreviations used for the thiazolium cations



Abbreviations	R	R′	R″
MMH	methyl	methyl	hydroxyethyl
EMH	ethyl	methyl	hydroxyethyl
EM	ethyl	methyl	-H
BMH	benzyl	methyl	hydroxyethyl
MBMH	4-methylbenzyl	methyl	hydroxyethyl
CBMH	4-chlorobenzyl	methyl	hydroxyethyl
DCBMH	2,6-dichlorobenzyl	methyl	hydroxyethyl
NBMH	4-nitrobenzyl	methyl	hydroxyethyl
NBM	2-nitrobenzyl	methyl	-H
EaB	ethyl		

Reactions were performed either by mixing neutral thiazolium solutions with basic buffers or by mixing thiazolium solutions at pH = 12.0 with less basic or acidic buffer solutions. Equal volumes of the reactants were mixed. All experiments were performed at 293 K using a concentration of 5×10^{-5} mol dm^{-3} thiazolium salt.

Reactions were followed by observing the optical absorption of the thiazolium salts. (The absorption maxima are at wavelengths of 245–265 nm.) A stopped-flow equipment with dual beam detection was used for relaxation times $\tau \leqslant 100$ seconds, slower reactions were observed with either a double beam spectrophotometer (Uvikon 860, Kontron) or a diode array spectrophotometer (HP 8452 A), which was equipped with a stopped-flow unit.

All kinetic measurements were performed under pseudo-first order conditions and could be fitted to eqn. (1) for one ($X_f = 0$) or two superimposed relaxation effects. X_i and τ_i are called relaxation amplitudes and relaxation times, respectively.

$$A_t = X_f \cdot e^{-t/\tau_f} + X_s \cdot e^{-t/\tau_s} + A_e \qquad (1)$$

Results

At equilibrium only cation $\mathbf{1}^+$ and anion $\mathbf{4}^-$ exist in significant concentrations, *i.e.* from spectrophotometric titrations we obtain only the equilibrium constant K_{41}, see eqn. (2), where *f* is

Table 2 Rate and equilibrium constants for the hydrolysis of thiazolium cations

	MMH	EMH	BMH[1]	MBMH	CBMH	DCBMH	NBMH	NBM	EM	EaB
pK_m	10.60	10.50	10.00	10.05	9.85	9.60	9.40	8.85	9.55	6.65
pK_{21}	13.0	13.0	12.3	12.4	12.3	11.5	12.0	11.0	12.5	9.2
pK_{32}	13.4	13.5	13.3	12.9	12.7	12.2	12.3	11.2	12.6	9.9
pK_{26}	6.7	6.5	6.1	7.1	6.4	6.7	7.4	6.7	6.0	4.7
pK_{43}	7.1	7.3	6.8	7.0	7.0	7.1	6.9	6.4	7.0	3.6
$\log k_1^H$*	−8.08	−8.22	−8.00	−7.34	−7.92	−7.15	−6.62	−6.60	−8.92	−5.85
$\log k_1^{OH}$**	0.57	0.32	0.88	0.79	0.91	0.94	1.17	1.48	0.48	3.62
$\log k_{-2}$*	−0.32	−0.40	−0.26	−0.48	−0.36	−0.65	−0.23	−0.95	−1.10	0.12

* s^{-1}; ** $dm^3\ mol^{-1}\ s^{-1}$.

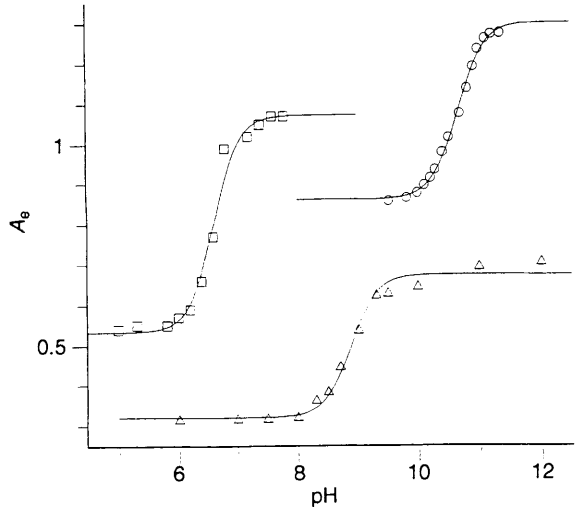


Fig. 1 Spectrophotometric titrations: absorbance as function of pH. (○) MMH, 226 nm, (△) NBM, 240 nm, (□) EaB, 276 nm. The curves are calculated with the constants given in Table 2.

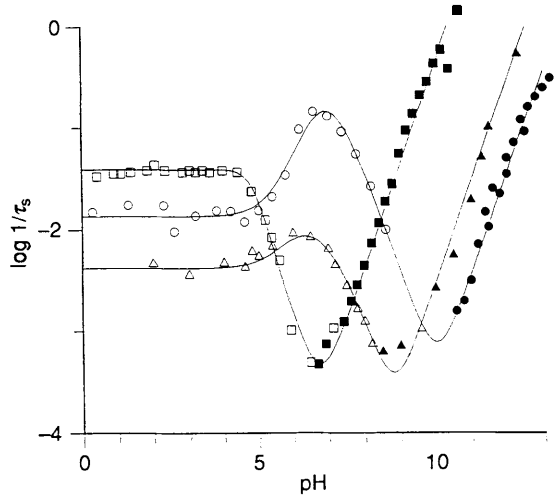


Fig. 2 Log $1/\tau_s$ as function of pH both for forward (●, ▲, ■) and backward (○, △, □) reaction; (○) MMH, (△) NBM and (□) EaB. The curves are calculated with the constants given in Table 2.

the activity coefficient of monovalent ions. Since two protons are involved in the reaction from $\mathbf{4}^-$ to $\mathbf{1}^+$, we observe a steep step in the titration curve at pH = pK_m, where the mean pK value is defined in eqn. (3). The results obtained for pK_m are summarized in Table 2, and in Fig. 1 the titration curves are shown for MMH, NBM and EaB. (For those compounds the experimental results differ most strongly from each other.)

$$K_{41} = c_4 \cdot c_H^2 \cdot f^2/c_1 \quad (2)$$

$$pK_m = -0.5 \cdot \log (K_{41}\ dm^6\ mol^{-2}) \quad (3)$$

A first set of kinetic experiments was performed by mixing neutral solutions of thiazolium salts with basic buffer solutions (pH ≥ pK_m − 0.5), where the cation $\mathbf{1}^+$ reacts with two hydroxy ions to form the anion $\mathbf{4}^-$ under the release of one water molecule (forward reaction). In these experiments the absorbance changes in a single relaxation effect from A_i to A_e. Relaxation times are plotted as function of the final pH in Fig. 2.

In a second set of experiments basic solutions of thiazolium salts (pH > pK_m + 1) were mixed with less basic buffer solutions (pH ≤ pK_m + 0.5), that means the anion $\mathbf{4}^-$ reacts with water or protons forming cation $\mathbf{1}^+$ (backward reaction). In these experiments up to three superimposed relaxation effects are observed, as indicated in Fig. 3. The absorbance changes from A_i to A_1 in a very fast reaction, which cannot be followed by the stopped-flow technique, and the further change in absorbance is described by eqn. (1). The solutions are mixed at time t = 0, and the symbols have the following meaning: A_e absorbance at equilibrium; $A_2 = (A_e + X_s)$ absorbance calculated by reextrapolating the slow relaxation effect to t = 0 according to eqn. (1) with X_f = 0; $A_1 = (A_e + X_s + X_f)$ absorbance obtained by reextrapolating both the slow and the fast relaxation effect to t = 0 according to eqn. (1); A_i initial absorbance, calculated from the solutions to be mixed; τ_s relaxation time of the slow effect; τ_f relaxation time of the fast effect. For the three compounds chosen τ_s and τ_f are plotted in Figs. 2 and 4, and A_1 and A_2 are plotted in Figs. 5 and 6, respectively. Details of the measurements and all experimental results are given in refs. 8 and 9.

For the backward reaction we observe three steps in the change of absorbance with time, and we can attribute these steps to reactions in Scheme 1 in the following way. Before mixing the solutions the thiazolium salt exists completely as anion $\mathbf{4}^-$. During the very fast change from A_i to A_1, equilibrium is reached between $\mathbf{4}^-$ and **3** according to the dissociation constant K_{43}, see eqn. (4). This means that this constant is obtained by fitting the titration curve for A_1 to a single dissociation reaction, see Fig. 6. During the fast change in absorbance from A_1 to A_2 equilibrium is reached between $\mathbf{4}^-$, **3** and **2**, which may be protonated to $\mathbf{6}^+$ with diffusion controlled speed. This fast process involves the two dissociations from $\mathbf{4}^-$ to (**3**, **2**) and from (**3**, **2**) to $\mathbf{6}^+$. However, in Fig. 5 we observe only a single dissociation step which is attributed to the dissociation constant $K_{4,32}$, eqn. (5).

$$K_{43} = c_4 \cdot c_H \cdot f^2/c_3 \quad (4)$$

$$K_{4,32} = \frac{c_4 \cdot c_H \cdot f^2}{(c_2 + c_3)} = \frac{K_{43} \cdot K_{32}}{1 + K_{32}} \quad (5)$$

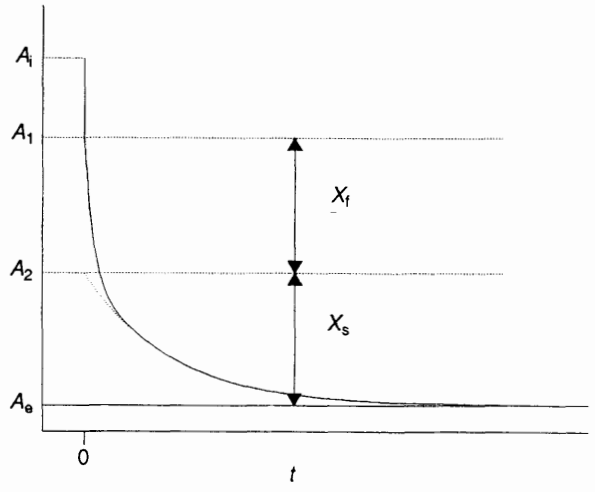


Fig. 3 Change in absorbance A during the backward reaction, schematically

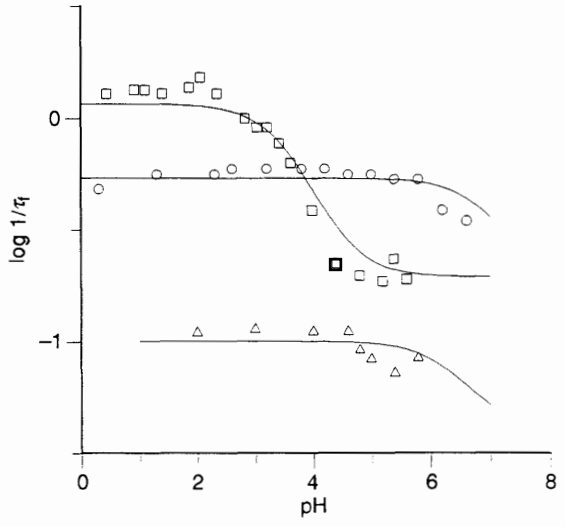


Fig. 4 Log $1/\tau_f$ as function of pH for the backward reaction; (○) MMH, (△) NBM and (□) EaB. The curves are calculated with the constants given in Table 2.

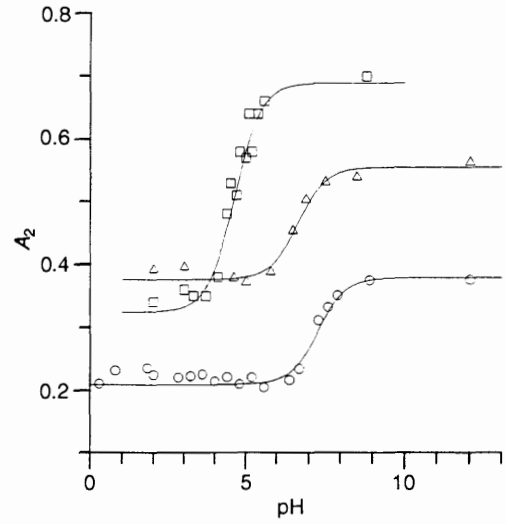


Fig. 5 Absorbance A_2 as function of pH measured for the backward reaction: (○) MMH, 258 nm; (△) NBM, 260 nm; (□) EaB, 248 nm. The curves are calculated with the constants given in Table 2.

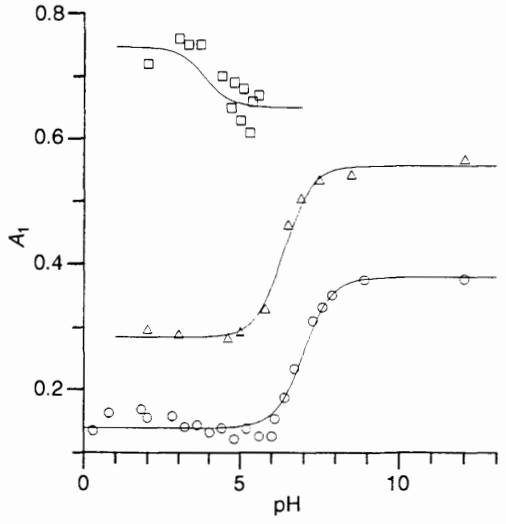
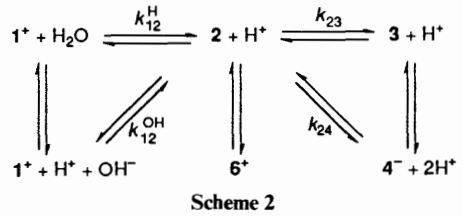


Fig. 6 Absorbance A_1 as function of pH measured for the backward reaction: (○) MMH, 258 nm; (△) NBM, 260 nm; (□) EaB, 248 nm. The curves are calculated with the constants given in Table 2.

For the discussion of the relaxation times we rewrite the reaction scheme as Scheme 2, where for step $\mathbf{1}^+$ to **2** we introduce the two reaction paths for acidic and basic solution.



Scheme 2

For this scheme, the relaxation times attributed to the fast step (**3**, $\mathbf{4}^-$ to **2**) and the slow step (**2**, **3**, $\mathbf{4}^-$, $\mathbf{6}^+$ to $\mathbf{1}^+$) are determined by eqns. (6) and (7), respectively.[1]

$$\frac{1}{\tau_f} = k_{-2} \cdot \left(\frac{K_{32}}{1 + K_{62} \cdot c_H} + \frac{c_H \cdot f^2}{c_H \cdot f^2 + K_{43}} \right) \quad (6)$$

With $k_{-2} = (k_{23} + k_{24})/K_{32}$

$$\frac{1}{\tau_s} = (k_{12}^{OH} \cdot K_W + k_{12}^H \cdot c_H) \cdot \left[(c_H)^{-1} + \left(K_{21} + \frac{K_{41}}{c_H \cdot f^2} + K_{61} \cdot c_H + K_{31} \right)^{-1} \right] \quad (7)$$

K_{62}, K_{43}, K_{21} and K_{31} are the acid dissociation constants, K_{41} and K_{32} are defined by eqns. (2) and (5) respectively, and K_{61} is given by eqn. (8). (It is obvious, that K_{41}, K_{32} and K_{61} can be calculated from the four dissociation constants.)

$$K_{61} = c_6/c_1 \quad (8)$$

The slow relaxation time depends on the proton concentration in a very characteristic manner. Therefore the fitting of eqn. (7) to the experimental data yields reliable values for the five constants k_{12}^H, k_{12}^{OH}, K_{41}, K_{61} and $(K_{21} + K_{31})$. The dependence of the fast relaxation time is only weakly pronounced, and the fitting of eqn. (6) allows us to determine k_{-2} and to estimate a lower limit for K_{43} with the exception of the data for EaB, from which k_{-2}, K_{43} and K_{32} are evaluated. Thus for all compounds K_{41} is obtained in two independent ways from the equilibrium titration and the slow relaxation time.

Analogously $K_{4,32}$ is obtained from both the relaxation time and the amplitude of the slow relaxation effect. The good agreement between the independently calculated values for the two constants (and additionally for K_{43} in the case of EaB) supports strongly the validity of Scheme 2. Details of the fitting are described in the previous paper.[1] Table 2 summarizes all constants determined by this evaluation. The curves in Figs. 1, 2, 4, 5 and 6 are calculated with these constants, indicating an excellent agreement between measurements and calculated values.

From the results of the equilibrium measurements and the stopped-flow experiments all equilibrium constants involved in Scheme 1 could be evaluated. In Fig. 1 the titration curves indicate a two proton process, for which the mean dissociation constant is obtained with an accuracy of $\delta pK_m = \pm 0.1$. For the calculation of the single dissociation constants both relaxation amplitudes have to be evaluated as well as the slow relaxation time. Therefore the error in these constants is larger and can be estimated to be $\delta pK_{21} = \pm 0.3$, $\delta pK_{31} = \pm 0.5$, $\delta pK_{43} = \pm 0.3$ and $\delta pK_{26} = \pm 0.3$. From the linear dependence of τ_s from c_{OH} at high pH values we obtain k_{12}^{OH} with an accuracy of $\pm 15\%$, whereas the constant value of τ_s at low pH values yields the product $k_{12}^{H} \cdot K_{16}$, and thus the inaccuracy of K_{26} determines the error in k_{12}^{H}. Finally the constant value of τ_f yields k_{-2} with an error of $\pm 20\%$.

Discussion

In a previous study it was shown that Scheme 1 describes the hydrolysis of the thiazolium cation BMH and equilibrium, and rate constants of the reactions involved were determined. The aim of the present work was to investigate whether Scheme 1 also describes correctly the hydrolysis of the thiazole ring of thiamine. For thiamine the substituent R is a pyrimidine moiety linked by a methylene bridge to the thiazole nitrogen atom, and the amino group at the pyrimidine allows further reactions, namely protonation and formation of the so-called yellow form by ring closure between the pyrimidine moiety and the thiazole ring. In order to avoid the complications due to these further reactions, we studied the hydrolysis of the thiazolium salts MMH, EMH, MBMH, CBMH, DCBMH and NBMH, where the pyrimidine ring is replaced by other substituents.

The results summarized in Table 2 indicate that the change of the substituent does not influence significantly the rate and equilibrium constants for the hydrolysis reactions. Thus we may assume that similar results will be obtained for thiamine. The dependence of the rate and equilibrium constants on the nature of the substituent R is too weak (and the error widths are too large) to justify a discussion along the lines of a free energy relationship.

The experimental results presented in the Figs. indicate that a change of the substituents R′ and R″ does not influence the reaction mechanism either, even for EaB, where R′ and R″ are replaced by an annelated benzene ring and where the constants differ strongly from those obtained for the other compounds. Furthermore we would only like to hint to the fact that K_{21} is relatively small for DCBMH and NBM. This may be due to an interference between the *ortho*-chloro or *ortho*-nitro group of the substituent with that carbon atom of the thiazole ring where the hydrolysis reaction starts.

Summarizing it may be said, that Scheme 1 describes correctly the hydrolysis of thiazolium cations. Therefore it will be the basis for the discussion of a new investigation of the hydrolysis of thiamine.

References

1 I. Heiber-Langer, I. Winter and W. Knoche, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1551.
2 R. Breslow, *J. Am. Chem. Soc.*, 1957, **79**, 1762.
3 (*a*) M. W. Washabaugh and W. P. Jencks, *Biochem.*, 1988, **27**, 5044; (*b*) M. W. Washabaugh and W. P. Jencks, *J. Am. Chem. Soc.*, 1989, **111**, 674, 683.
4 H. Stetter, *Angew. Chem.*, 1976, **88**, 685.
5 H. T. Clarke, *J. Am. Chem. Soc.*, 1944, **66**, 652.
6 R. A. Robinson and R. H. Stokes, *Electrolyte Solutions*, Butterworth, London, 2nd edn. 1959.
7 C. W. Davies, *Ion Association*, Butterworth, London 1962.
8 I. Heiber-Langer, Dissertation, Bielefeld, 1990.
9 S. Barrabass, Dissertation, Bielefeld, 1992.

Paper 3/04166B
Received 16*th July* 1993
Accepted 22*nd September* 1993