Rapid Hydrogen Exchange at the Acetyl (C2) Methyl Group of Acetylthiamin Pyrophosphate

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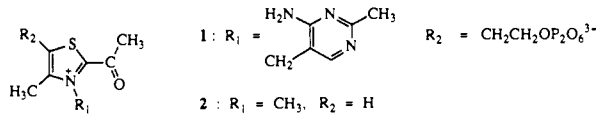
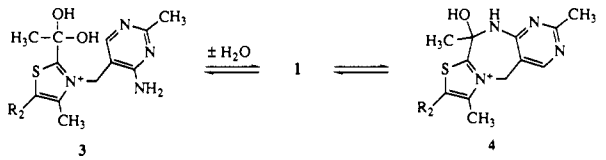
We have discovered that the acetyl methyl hydrogens of 2-acetylthiamin pyrophosphate (AcTPP, **1**) and 2-acetyl-3,4-dimethylthiazolium ion (**2**) rapidly exchange with water. We present these preliminary studies, which suggest that the electrophilic thiazolium ring enhances the ionization rate.



AcTPP exists in aqueous solution in equilibrium with its hydrate **3** and internal carbinolamine adduct **4**.[1] The equilibrium constants for the interconversion of the forms in D_2O are K_1 = [**4**]/[**1**] = 0.5 and K_2 = [**3**]/[**1**] = 1.1 (pD = 13.5, 25 °C). The hydration



3 4

equilibrium constant for **2** in D_2O was calculated[3] as K_h = 1.3 ± 0.1 (pD = 1.3, I = 0.05, 25 °C).[2] In H_2O at higher ionic strength, K_h = 0.83 (pH = 2, I = 0.5).[2]

In the course of investigating the equilibria of AcTPP at increasing pD (>4), we observed a decrease of the proton NMR signal for the C2 methyl protons corresponding to each form of AcTPP. No changes in these signals could be detected at pD 2.[1a] We also observed the disappearance of the C2 methyl signals of the hydrate and ketone forms of 2-acetyl-3,4-dimethylthiazolium ion (**2**). As these signals diminished, they gave rise to multiplet signals 0.016 and 0.034 ppm upfield from the methyl signals. These observations indicated that deuterium exchanges into the C2 methyl groups of these molecules, and the multiplets could be assigned to the species-CH_2D and-CHD_2. The observation of deuterium incorporation at pD 4 was surprising and prompted further studies.

The incorporation of deuterium into 2-acetyl-3,4-dimethylthiazolium iodide and AcTPP takes place simultaneously with their hydrolysis to acetic acid and the deacylated thiazolium ions. Hydrolysis and exchange take place at comparable rates. Analysis of the deuterium exchange can be made on the basis that interconversion of the hydrate and ketone (and carbinolamine for AcTPP) forms are fast relative to exchange or hydrolysis, which is validated by the constant proportions of the hydrate, ketone, and carbinolamine throughout the course of these reactions. Analysis for deuterium exchange also depends on the exchange and hydrolysis being irreversible and on the secondary deuterium isotope effect on the hydrolysis being small, which is very likely to be the case. The rates for deuterium incorporation into these molecules can be measured by ^{1}H NMR.[4]

Table I. Rate Constants for Exchange of Deuterium into C2 Methyl Groups of AcTPP and 2-Acetyl-3,4-dimethylthiazolium Iodide in D_2O

		$k_{ex}/10^{-5}$ s^{-1} [a]		
substrate	pD	hydrate	ketone	carbinolamine
AcTPP	4.01[b]	5.9	5.9	4.2 (k_{av} = 5.3 ± 0.8)
	4.15[c]	12.2	7.9	9.6 (k_{av} = 9.9 ± 2.2)
2[d]	4.10[b]	2.31, 2.06	2.50, 2.03	(k_{av} = 2.23 ± 0.23)
	4.54[b]	3.08	3.12	(k_{av} = 3.10 ± 0.03)
	4.77[e]	4.61	5.21	(k_{av} = 4.91 ± 0.43)

[a] Hydrogen exchange for a single proton measured by ^{1}H NMR.[4] [b] 50 mM Na oxalate buffer at 25 °C. [c] 50 mM Na phosphate buffer at 24 °C. [d] 2-Acetyl-3,4-dimethylthiazolium iodide. [e] 41 mM Na oxalate buffer at 25 °C.

Rate constants for the exchange of deuterium into AcTPP and 2-acetyl-3,4-dimethylthiazolium ion are included in Table I. The rate constants for exchange of the three forms of AcTPP are identical within error, as they are for the two forms of 2-acetyl-3,4-dimethylthiazolium ion. This confirms that interconversions of the keto and hydrate forms are fast relative to the exchange reactions. The rate constants for exchange increase with pD, and exchange is not observed at pD ≤ 2. Therefore, the exchange reaction is not acid catalyzed but is base catalyzed and presumably proceeds through an enolization mechanism.

We also measured the enolization rate of 2-acetyl-3,4-dimethylthiazolium iodide in dilute oxalate buffer by iodine scavenging.[6] The rate constants at 25 °C were found to be (6.73 ± 0.22) × 10^{-5} s^{-1} at pD 4.17 (46 mM Na oxalate) and (22.8 ± 1.8) × 10^{-5} s^{-1} at pD 4.77 (36 mM Na oxalate). These compared well with the statistically corrected values ($3k_{ex}$) from Table I. We did not observe acid catalysis of enolization but were able to use the iodine scavenging method to estimate the rate constant for the uncatalyzed enolization, which was found to be k_o^E = (3.8 × 0.5) × 10^{-6} s^{-1}. The effects of buffers such as acetate, pyridine, and 2,6-lutidine showed that general bases catalyze enolization but general acids are ineffective. The rate constants extrapolated to zero buffer concentrations allowed the second-order rate

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(3) For **2**: δ(ppm, hydrate in D_2O) 1.86 (s, 3 H), 2.51 (s, 3 H), 4.12 (s, 3 H), 7.69 (s, 1 H); δ (ppm, ketone in D_2O) 2.58 (s 3 H), 2.81 (s, 3 H), 4.14 (s, 3 H), 8.02 (s, 1 H). The values for forms 1, 3, and 4 of AcTPP are in ref 1a.

(4) Consider the following kinetic scheme for exchange of the acetyl methyl protons of AcTPP or 2-acetyl-3,4-dimethylthiazolium ion (R-CH_3):

$$\text{R-CH}_3 \xrightarrow{k_1} \text{R-CDH}_2 \xrightarrow{k_2} \text{R-CD}_2\text{H}$$

At any time t, let [R-CH_3] = a and [R-CDH_2] = b; and at t = 0, a = a_o and b = 0. For these consecutive first-order reactions, the differential equations and their solutions are given by:

$$-da/dt = k_1 a \qquad a = a_o \exp(-k_1 t)$$

$$db/dt = k_1 a_o \exp(-k_1 t) - k_2 b \qquad b = k_1 a_o/(k_2 - k_1)[\exp(-k_1 t) - \exp(-k_2 t)]$$

The rate constant k is defined for the exchange of a single proton; therefore, the values of k_1 and k_2 are $3k$ and $2k$, respectively. Substitution of these values into the equations above give: $a = a_o \exp(-3kt)$ and $b = 3a_o[\exp(-2kt) - \exp(-3kt)]$. From these expressions, it follows that the ratio $a/(a+b/3) = \exp(-kt)$; that is [R-CH_3]/[[R-CH_3] + R-CDH_2]/3] = $\exp(-kt)$. The normalized integral ratios were measured by ^{1}H NMR spectroscopy as a function of time to the extent of 70-80% exchange of one proton. The normalized integral ratios fitted well to the expression for a single exponential decay. Hydrolysis to acetic acid gradually decreased the concentrations of AcTPP and **2** in the course of exchange but did not interfere with the measurements of normalized integral ratios for the residual compounds.

(5) (a) Hydroxide ion catalytic coefficient of AcTPP is estimated by $k_{ex}(1 + K_h)/\log^{-1}(pD - 14.81)$. (b) Rate constant for hydroxide-catalyzed ionization of acetone is 0.224 ± 0.003 M^{-1} s^{-1} (Chiang, Y.; Kresge, A. J.; Morimoto, H.; Williams, P. *G. J. Am. Chem Soc.* **1984**, *114*, 3981-3982).

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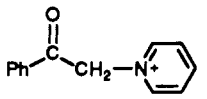
constant for hydroxide-catalyzed enolization at 25 °C to be calculated as $k_{OH}^{E} = (1.2 \pm 0.2) \times 10^5\ M^{-1}\ s^{-1}$.

The exchange reaction likely takes place through the rate-limiting loss of a proton to produce an enolate ion, which undergoes deuteration in D_2O. If we assume that the ionizations of AcTPP and **2** are mostly OD-catalyzed under our conditions, the rates seem quite fast relative to the ionizations of simple alkyl carbonyl compounds in aqueous solutions of pD = 4. For example, AcTPP ionizes on the order of 10^7 faster than acetone.[5] Most carbon acids such as aldehydes, ketones, and esters undergo ionization abnormally slowly, presumably owing to the rehybridization required to stabilize most carbanions.[6] The rate of enolate ion formation from a carbonyl compound is related to the carbon acidity.[6] The fast rate of exchange by AcTPP could be attributed to a low pK_a of the acetyl group, to intramolecular catalysis by the aminopyrimidinyl or the ethyl pyrophosphate groups, or to structural factors causing the molecule to ionize as a more nearly "normal" acid than other ketones. Comparing the exchange rates of AcTPP (pD = 4.15) and **2** (pD = 4.10), an enhancement factor of about 4 can be attributed to the aminopyrimidinyl and ethyl pyrophosphate groups of AcTPP (Table I). Therefore, the major rate enhancement is brought about by the thiazolium ring.

The second-order rate constant for hydroxide-catalyzed enolization of **2** is 5–6 orders of magnitude larger than that for acetaldehyde or a collection of methyl ketones, including acetone and *p*-nitroacetophenone.[5,6] Comparison of k_{OH}^{E} for **2** with the linear correlation of k_{OH}^{E} versus pK_a compiled by Keeffe and Kresge for carbon acids[6] would indicate a pK_a of 3.7 for **2**. This is an unrealistically low value because all examples of carbonyl compounds that exhibit pK_a values below 13 have at least one additional electron-withdrawing substituent on the α-carbon atom. For example, the pK_a of compound **5** is 10.9. The fast exchange rates for AcTPP and **2** are unlikely to be due to extremely low pK_as.

We believe that the largest factor contributing to the fast enolization of AcTPP and **2** is the through-space electrostatic effect of the positive charge in the thiazolium ring. Fast rates of enolization have been reported for other cationic ketones;[7] for example, **5** undergoes enolization at a rate ($k_{OH}^{E} = 1.8 \times 10^5\ M^{-1}\ s^{-1}$)[7b,c] that is comparable to that for **2**, and **6** undergoes very fast water-catalyzed and general-base-catalyzed enolization.[7d] Ionizations of the C2 protons of thiamine pyrophosphate and 3,4-dimethylthiazolium ion also proceed rapidly.[8]



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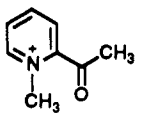

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Rate enhancement in the ionization of carbon acids and the enolization of carbonyl compounds is poorly understood. In this connection, electrophlic catalysis of the enzymatic enolization of mandelate has recently been discussed.[9] Our results show that fast rates of base-catalyzed enolization can be brought about by the through-space electrostatic effect of a nearby positive charge.

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