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Thiazolium C(2)-Proton Exchange: Structure–Reactivity Correlations and the pK_a of Thiamin C(2)–H Revisited†

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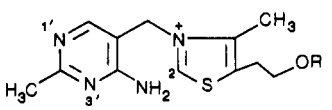
ABSTRACT: Rate constants for C(2)-proton exchange from thiamin, *N*(1′)-methylthiamin, and several 3-substituted-4-methylthiazolium ions catalyzed by D_2O and deuterioxide ion were determined by ^1H NMR at 30 °C and ionic strength 2.0 M. Values of pK_a for the thiazolium ions, including thiamin itself, were found to be in the range pK_a = 17–19; the pK_a values for N(1′)-protonated thiamin and free thiamin C(2)-H in H_2O are 17.7 and 18.0, respectively. The pK_a value for N(1′)-protonated thiamin was calculated from the observed rate constant for the pD-independent reaction with D_2O after correction for a secondary solvent deuterium isotope effect of k_{H_2O}/k_{D_2O} = 2.6. The pK_a value for free thiamin was calculated from the rate constant for catalysis by OD^- after correction by a factor of 3.3 = 8/2.4 for an 8-fold negative deviation of k_{OD} from the Brønsted plot of slope 1.0 for general base catalysis and a secondary solvent isotope effect of k_{OD}/k_{OH} = 2.4. Values of $k_{-a} = 2 \times 10^{10}$ and 3×10^9 M^{-1} s^{-1} were assumed for diffusion-controlled protonation of the C(2) ylide in the reverse direction by H_3O^+ and H_2O, respectively. The Hammett ρ_I value for the exchange reaction catalyzed by deuterioxide ion or D_2O is 8.4 ± 0.2. There is no positive deviation of the rate constants for free or N(1′)-substituted thiamin analogues in either Hammett correlation. This shows that the aminopyrimidinyl group does not provide significant intramolecular catalysis of nonenzymic C(2)-proton removal in the coenzyme.

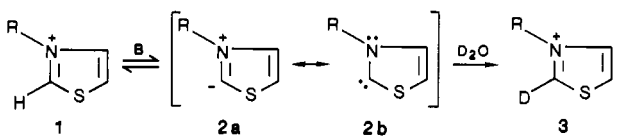
Thiamin pyrophosphate (TPP)[1] (**1a**) contains substituted pyrimidine and thiazolium heterocycles and is a coenzyme for the decarboxylation of α-keto acids, the formation of α-ketols, and transketolase reactions (Krampitz, 1969; Schowen & Schellenberger, 1987). The catalytic action of thiamin in both



1a, R = $P_2O_6H_3$
b, R = H

thiamin-dependent enzyme reactions and nonenzymic model reactions results from the base-catalyzed abstraction of the C(2) proton leading to the formation of the very reactive thiazolium ylide (**2**, Scheme I), which is not only a potent carbon nucleophile but also a reasonably stable leaving group (Breslow, 1962).

Scheme I



1 **2a** **2b** **3**

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[1] Abbreviations: TPP, thiamin pyrophosphate; Me_2SO, dimethyl sulfoxide.

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Transfer of the C(2) proton of thiamin and thiazolium ions has been reviewed (Metzger, 1984; Kluger, 1987). Proton transfer from carbon differs from proton transfer from the electronegative atoms of "normal" acids in that it is usually much slower and is generally believed to occur directly, rather than through a solvent molecule. This slow proton transfer has been attributed to changes in electron delocalization and hybridization accompanied by changes in the lengths and angles of bonds to heavy atoms, poor hydrogen bond donor–acceptor properties of carbon, and solvent reorganization in the rate-limiting transition state (Bell, 1973; Kresge, 1975; Hine, 1977; Bernasconi, 1982; Vinogradov & Linnell, 1971). Thiamin is an especially simple carbon acid that requires little or no delocalization or change in bond lengths and angles of heavy atoms upon ionization; ionization gives an electron lone pair in an sp^2 hybridized orbital that cannot be stabilized by $p-\pi$ delocalization. We are interested in the rate of thiamin C(2)-proton exchange, which is required for functioning of the coenzyme.

Several groups have evaluated the rate of thiamin C(2)-proton exchange and found that the enzyme-mediated reaction of thiamin with pyruvate is at least 10^4 times faster than the maximum rate possible in the nonenzymic reaction. The scale of this rate ratio suggests the presence within the enzyme of a higher concentration of thiamin C(2) ylide than can be realized in water. Thus, a major role of thiamin-dependent enzymes might be to change the relative thermodynamic stabilities of thiamin and its ylide (Crosby & Lienhard, 1970; Kemp & O'Brien, 1970).

An alternate explanation for the relatively fast enzymic proton transfer was offered by Schellenberger and Petzold, who proposed that the exocyclic 4′-amino group functions as an intramolecular base catalyst for C(2)-proton abstraction in the enzymic and nonenzymic exchange reactions, respectively (Schellenberger, 1967; Petzold, 1976). Gallo and Sable demonstrated that C(2)-proton exchange rates for several 3-R-4-methylthiazolium cations, including thiamin analogues, correlate with the acidities of a series of corresponding primary ammonium ions. They concluded that these C(2)-proton exchange rates reflect differing electron-withdrawing abilities of the substituents attached to N(3), as noted previously by others (Breslow, 1958; Yount & Metzler, 1959), and appear not to be related to intramolecular base catalysis by the exocyclic 4′-amino group (Gallo & Sable, 1976). In spite of this earlier conclusion to the contrary, reports persist that the exocyclic 4′-amino group functions as an intramolecular general base catalyst in nonenzymic thiazolium C(2)-proton exchange (Petzold et al., 1982). Jordan has suggested that protonation or metal substitution at N(1′) ($pK_a \approx 5$) is required for the 4′-amino group to function as a catalyst [pK_a = 7.5–12.7, with N(1′) substituted] (Jordan & Mariam, 1978; Jordan et al., 1982).

The pK_a of thiamin C(2)–H, both in aqueous solution and on thiamin-dependent enzymes, is a critical unknown in studying reaction mechanisms involving this coenzyme. On the basis of rapid-reaction studies, Hopmann and Brugnoni have reported pK_a = 12.7 for the nonenzymic deprotonation of thiamin C(2)–H in aqueous solution (Hopmann & Brugnoni, 1973). This value is much lower than pK_a values in the range 17–20 that have been estimated for this deprotonation by others (Breslow, 1962; Crosby & Lienhard, 1970; Kemp & O'Brien, 1970; Kluger, 1987).

We have determined rate constants for catalysis by D_2O and OD^- of C(2)-proton exchange in thiamin and several related thiazolium ions and calculated C(2)-H pK_a values in the range pK_a = 17–19 from these rate constants; the pK_a values for N(1′)-protonated thiamin and free thiamin are 17.7 and 18.0, respectively. We have also examined the role of inductive effects on the rate of nonenzymic thiazolium C(2)-proton exchange in aqueous solution in order to evaluate the importance of intramolecular general base catalysis by the aminopyrimidinyl group of thiamin for proton abstraction. Our results confirm and extend the previous conclusion (Gallo & Sable, 1976) that the aminopyrimidinyl group has no effect, other than inductive, on the nonenzymic C(2)-proton exchange rate of thiamin.

Experimental Procedures

Materials. All chemicals were of analytical or reagent grade and were used without further purification unless otherwise stated. Water was glass distilled. Thiamin hydrochloride (**1b**), 4-amino-5-(aminomethyl)-2-methylpyrimidine dihydrochloride, 4-methylthiazole, alkyl halides, sodium deuterioxide, deuterium chloride, deuterium oxide, acetic-d_3 acid-d, and phosphoric acid-d_3 were purchased from Aldrich. Dimethyl-d_6 sulfoxide was purchased from Bio-Rad. All deuteriated compounds were ≥99 atom % D. Thiamin hydrochloride was recrystallized from methanol/ethanol: mp 242–243 °C dec. *N*(1′)-Methylthiaminium diiodide (**14**, see Table I) was prepared by N-methylation of thiamin mononitrate (Serva) with methyl iodide and recrystallized from methanol/diethyl ether: mp 218–220 °C dec (lit. 214–220 °C dec) (Jordan & Mariam, 1978). The synthesis of 3,4-dimethylthiazolium chloride (**4**) has been described (Crosby et al., 1970). The other thiazolium salts were prepared from 4-methylthiazole and an excess of the appropriate alkyl halide. The salts were crystallized from ethanol/diethyl ether unless stated otherwise.

3-Ethyl-4-methylthiazolium bromide (**5**): mp 173–174 °C [lit. 170–171 °C (Breslow, 1958)]; ^{13}C NMR δ 15.4, 16.7, 51.3, 123.7, 149.4, 159.3; ^1H NMR δ 1.60 (t, 3 H), 2.62 (s, 3 H), 4.5 (q, 2 H), 7.88 (s, 1 H), 9.90 (d, 1 H); IR ν_{max} 3440, 3040, 2985, 1580, 1480, 1450, 1425, 1395, 1385, 1365, 1320, 1305, 1215, 1155, 1105, 1055, 1015, 925, 855 cm^{-1}.

3-Propyl-4-methylthiazolium iodide (**6**): mp 146–147 °C; ^{13}C NMR δ 12.8, 15.7, 25.2, 57.4, 123.7, 149.4; ^1H NMR δ 0.92 (t, 3 H), 1.92 (q, 2 H), 2.57 (s, 3 H), 4.37 (t, 2 H), 7.8 (s, 1 H), 9.82 (d, <1 H due to proton exchange); IR ν_{max} 3410, 3080, 3045, 2960, 1565, 1475, 1460, 1425, 1390, 1365, 1355, 1210, 1155, 925, 900, 820 cm^{-1}.

3-(Carboxyethyl)-4-methylthiazolium bromide (**7**): mp 163–164 °C; ^{13}C NMR δ 15.3, 35.6, 50.9, 123.5, 149.5, 161.0, 176.4; ^1H NMR δ 2.65 (s, 3 H), 3.12 (t, 2 H), 4.78 (t, 2 H), 7.88 (s, 1 H), 9.98 (d, 1 H); IR ν_{max} 3380, 3135, 3110, 2960, 2910, 1735, 1585, 1480, 1430, 1400, 1380, 1340, 1290, 1230, 1220, 1205, 1150, 1120, 1065, 935, 900, 820 cm^{-1}.

3-Allyl-4-methylthiazolium bromide (**8**): mp 132–133 °C [lit. 132–134 °C (Breslow, 1958)]; ^{13}C NMR δ 15.6, 57.8, 124.1, 124.8, 132.0, 149.5, 160.4; ^1H NMR δ 2.60 (s, 3 H), 5.10 (m, 2 H), 5.48 (m, 2 H), 6.10 (m, 1 H), 7.90 (s, 1 H), 9.90 (d, <1 H); IR ν_{max} 3430, 3010, 2940, 1575, 1470, 1455, 1440, 1410, 1370, 1300, 1290, 1225, 1160, 1095, 1050, 1015, 995, 940, 925, 855, 820 cm^{-1}.

3-(Carboxymethyl)-4-methylthiazolium chloride (**9**) was prepared by hydrolysis of 3-(carbethoxymethyl)-4-methylthiazolium bromide (**12**) (3.5 g, 0.013 mol) in 25 mL of 1 M HCl under reflux for 18 h. After rotary evaporation to remove HCl, the pale yellow crude product was dissolved in water and converted to the chloride salt on a column of Dowex 1X8-100 anion-exchange resin in the chloride form. Rotary evaporation to remove solvent and recrystallization resulted in white needle crystals. The crystals were dried in vacuo over P_2O_5 at 75 °C:

mp >230 °C; yield = 1.6 g (63%); ^{13}C NMR δ 15.5, 56, 123.5, 150, 162.5, 172; ^{1}H NMR δ 2.55 (s, 3 H), 5.42 (s, 2 H), 7.9 (s, 1 H), 9.95 (d, 1 H); IR ν_{max} 3440, 3120, 3020, 2830, 2690, 2580, 2490, 1730, 1580, 1455, 1405, 1210, 1195, 1155, 990, 925, 885, 860, 855, 800, 775, 715, 615 cm^{-1}.

3-Benzyl-4-methylthiazolium chloride (**10**): mp 189–190 °C [lit. 191–192 °C (Haake et al., 1969)]; ^{13}C NMR δ 15.6, 59.1, 124.2, 131.1, 131.2, 132.3, 134.4, 149.5, 160.5; ^{1}H NMR δ 2.60 (s, 3 H), 5.78 (s, 2 H), 7.52 (m, 5 H), 8.0 (s, 1 H), 9.95 (d, <1 H); IR ν_{max} 3460, 3380, 3105, 2980, 2910, 1660, 1550, 1495, 1465, 1455, 1440, 1390, 1345, 1210, 1190, 1145, 1110, 1030, 965, 920, 880, 860, 790, 750, 700 cm^{-1}.

3-(4-Nitrobenzyl)-4-methylthiazolium bromide (**11**): mp 209–210 °C; ^{13}C NMR δ 16, 58, 124.5, 127.5, 132, 142, 149.5, 150.5, 161.5; ^{1}H NMR δ 2.55 (s, 3 H), 5.93 (s, 2 H), 7.58 (d, 2 H), 8.0 (s, 1 H), 8.32 (d, 2 H), 10.02 (d, 1 H); IR ν_{max} 3450, 3100, 3080, 3040, 3000, 2940, 1605, 1585, 1520, 1475, 1450, 1395, 1345, 1305, 1195, 1140, 1105, 1080, 1020, 920, 880, 855, 845, 815, 740, 695 cm^{-1}.

3-(Carbethoxymethyl)-4-methylthiazolium bromide (**12**): mp 141–142 °C; ^{13}C NMR δ 15, 16, 55.5, 66.5, 123.5, 150, 163, 169.5; ^{1}H NMR (Me_2SO-d_6) δ 1.25 (t, 3 H), 4.28 (q, 2 H), 5.68 (s, 2 H), 8.13 (s, 1 H), 10.3 (d, 1 H); IR ν_{max} 3420, 3070, 2990, 2920, 1740, 1570, 1450, 1390, 1370, 1240, 1220, 1190, 1020, 920, 875, 835, 795, 740 cm^{-1}.

3-(Cyanomethyl)-4-methylthiazolium Chloride (**13**). 3-(Cyanomethyl)-4-methylthiazolium iodide was prepared by gently refluxing 10.0 g (0.060 mol) of iodoacetonitrile with 2.0 g (0.020 mol) of 4-methylthiazole in 10 mL of dry benzene for 72 h. The yellow-brown solution gradually became red-brown and viscous. The benzene was removed by rotary evaporation, and the resulting red-brown oil was triturated 4 times with 20 mL of diethyl ether to obtain a brown granular solid. This solid was dissolved in about 400 mL of boiling ethanol, and fine yellow needle crystals formed upon cooling to room temperature from the clear orange mother liquor. The yellow crystals were collected on a sintered glass filter, rinsed once with ice-cold ethanol, and dried in vacuo over P_2O_5 at 50 °C: mp 161.5–162.5 °C, yield = 3.9 g (72%); ^{13}C NMR δ 14, 42, 113.5, 123, 148, 161; ^{1}H NMR δ 2.72 (s, 3 H), 5.75 (s, 2 H), 8.0 (s, 1 H), 10.15 (d, <1 H); IR ν_{max} 3110, 3080, 2980, 2920, 1560, 1450, 1445, 1405, 1375, 1370, 1185, 1160, 1120, 1035, 965, 910, 860, 820 cm^{-1}. As noted previously for a similar compound, the IR spectrum does not contain a nitrile absorption in the region of 2200 cm^{-1} (Yount & Metzler, 1959).

Anal. Calcd for $C_6H_7N_2SI$: C, 27.08; H, 2.65; N, 10.53; S, 12.05; I, 47.69. Found: C, 27.29; H, 2.68; N, 10.53; S, 12.46; I, 47.29. The iodide salt was converted to the chloride salt as described above: mp 222 °C dec.

Methods. Solution pH was measured with an Orion Model 701A pH meter and Radiometer GK2321C combination electrode standardized at pH 7.00 and 4.00 or 10.00 (25 °C) and at pH 6.99 and 4.01 or 9.96 (30 °C). The electrode was free of the anomalous ionic strength effects reported by Illingworth (1981). Noise-decoupled ^{13}C NMR spectra were recorded on a Varian XL-300 NMR spectrometer in D_2O. ^{1}H NMR spectra were recorded on the above instrument (kinetics) or on a Varian EM-390 NMR spectrometer (synthesis) in D_2O unless otherwise indicated. Sodium 3-(trimethylsilyl)propanesulfonate (DSS) was an internal standard for the ^{1}H NMR spectra (synthesis). The instrument external standard was used for the ^{13}C NMR spectra. Proton spin–spin coupling of C(2)–H ($\delta \approx 10$ ppm) to C(5)–H ($\delta \approx 8$ ppm) through sulfur was observed for all thiazolium salts studied. This coupling results in splitting of the C(2)–H peak to a doublet ($J = 3.0 \pm 0.5$ Hz) and an increased peak width for C(5)–H. IR spectra of compounds in KBr pellets were recorded on a Perkin-Elmer 683 spectrophotometer. Melting points are uncorrected. Elemental analysis was performed by Galbraith Laboratories.

Kinetics. Rate constants for thiazolium C(2)-proton exchange in D_2O catalyzed by deuterioxide ion and D_2O were determined by ^{1}H NMR spectroscopy as described by Haake et al. (1969). All reactions were carried out at 30 ± 1 °C. The probe temperature was measured by a thermocouple at the probe, which was calibrated by using the chemical shift difference of the methylene and hydroxyl protons of ethylene glycol [containing 0.03% (v/v) concentrated HCl] (Friebolin et al., 1979). The ionic strength was maintained at 2.0 M with NaCl. Buffers in D_2O were either 3×10^{-4}–2.7 M DCl, 10 mM sodium acetate-d_3 buffer (pD 3.5–6, from acetic-d_3 acid-d and NaOD), or 10 mM NaD_2PO_4 buffer (pD 6–7.5, from phosphoric acid-d_3 and NaOD). The exchange reaction was initiated by dissolving 0.0625 mmol of thiazolium salt in 0.5 mL of buffer, which was previously equilibrated at 30 °C, giving a final concentration of 0.125 M thiazolium salt in the reaction. For exchange rates where $15 \text{ s} \leq t_{1/2} \leq 60$ min, the solutions were rapidly transferred to 5-mm NMR tubes and placed in the spectrometer probe. When the magnet was shimmed on a test sample before the exchange reaction was initiated and the sample was placed in the probe, reactions with $t_{1/2} \approx 15$ s could be monitored with good precision (≤±5%). Integrated areas were measured for the C(2)–H signal and compared to those for the C(5)-H signal (as nonexchanging internal standard) as a function of time. For exchange reactions with $t_{1/2} > 60$ min, the NMR tube was placed in a constant-temperature bath (30 ± 0.2 °C), and the tube was removed for about 5 min every 6–24 h in order to measure the integrated areas of the C(2)–H and C(5)–H signals. The pseudo-first-order rate constants were usually obtained from semilogarithmic plots of A_2/A_5 against time, where A is the integrated area of the C(2)–H or C(5)–H signal, respectively, and from the relationship $k_{obsd} = 0.693/t_{1/2}$. These plots were linear for $> 3t_{1/2}$ with 10–15 time points. Pseudo-first-order rate constants for exchange reactions with $t_{1/2} > 1$ month were obtained by linear regression analysis using eq 1 with correlation coefficients greater than 0.990. Typical percents of reaction over which D_2O-catalyzed exchanges were followed were ≤5% (**4**), 25% (**11**, **15**), and 70% (**13**). Where duplicate determinations of k_{obsd} were made, they agreed within ±5% of the average value.

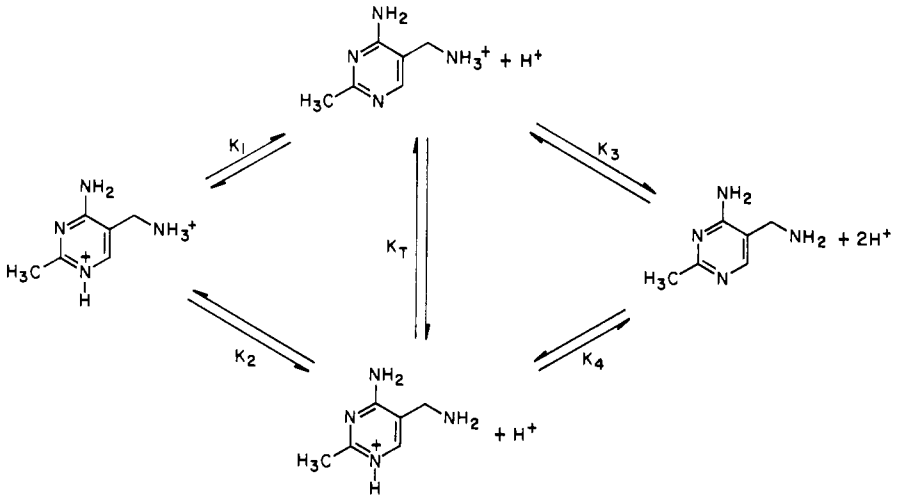
$$\ln (A_2/A_5) = k_{obsd}t + C \quad (1)$$

Measurements of pH were made at 30 ± 0.2 °C on the buffered solutions of the thiazolium salts after exchange had occurred. The value of pD was obtained by adding 0.40 to the observed pH of solutions in D_2O (Glasoe & Long, 1960). On the basis of measurements of pH at known concentrations of hydroxide ion at 30 °C and 2.0 M ionic strength, maintained with KCl, eq 2 was used to calculate the concentration of deuterioxide ion at any pD. This equation includes the ion product of deuterium oxide at 30 °C (Covington et al., 1966).

$$[OD^-] = 1.18 \times 10^{(pD-14.70)} \quad (2)$$

Second-order rate constants for deuterioxide ion catalyzed exchange were obtained from the slopes of plots of k_{obsd} against deuterioxide ion concentration containing four or more data points. Buffer bases do not significantly contribute to k_{obsd} under these reaction conditions and were neglected. The

Scheme II



second-order rate constant for D_2O-catalyzed exchange was calculated by dividing the observed pD-independent, buffer-independent pseudo-first-order rate constant by 55.1 M, the concentration of D_2O at 30 °C (Millero et al., 1971).

pK_a's of 4-Amino-5-(aminomethyl)-2-methylpyrimidine Dihydrochloride. The amine was dried in vacuo over P_2O_5 at 30 °C to constant weight before use. A 5.00×10^{-3} M solution of the amine in freshly boiled water was titrated potentiometrically with 0.1003 M KOH at 25.0 °C according to the method of Albert and Serjeant (1984). Solutions were kept under an argon atmosphere. Data were analyzed by assuming that the two ionization steps (K_1 and K_3 in Scheme II) overlap and a correction was applied to reflect changing ionic strength during the titration (Albert & Serjeant, 1984). Duplicate determinations gave $pK_1 = 5.07 \pm 0.02$ and $pK_3 = 8.58 \pm 0.03$. These pK_a values agree reasonably well with literature values for pK_1 [4.85 (Zoltewicz & Sridharan, 1978)] and pK_3 [8.01 (Zoltewicz & Sridharan, 1978), 8.4 (Yount & Metzler, 1959), and 8.6 (May & Sykes, 1966)]. $pK_a^{N1'} = 5.5$ for the aminopyrimidinyl group on thiamin in D_2O was estimated from $pK_a^{N1'} = 5.0$ in H_2O (Suchy et al., 1972; Gallo & Sable, 1975; Jordan & Mariam, 1978) and $\Delta pK_a = 0.5$ for the solvent isotope effect on the ionization of weak acids (Schowen & Schowen, 1982).

Estimation of σ_I for the Aminopyrimidinyl Group of Thiamin. We estimate the pH-dependent σ_I values for the 2-methyl-4-amino-5-methylenepyrimidinyl group on the basis of equilibria for 4-amino-5-(aminomethyl)-2-methylpyrimidine shown in Scheme II and a correlation (eq 3) of the pK_a values

$$pK_a^{RCH_2NH_3^+} = -8.6\sigma_I + 10.1 \quad (3)$$

for primary ammonium ions, $RCH_2NH_3^+$, with the σ_I inductive parameter for R (Fox & Jencks, 1974). Acidities of $RCH_2NH_3^+$ have previously been correlated with thiazolium C(2)-proton exchange rates (Yount & Metzler, 1959; Gallo & Sable, 1976), rates of deprotonation of 2,4-dimethylthiazolium ions (Zoltewicz & Sridharan, 1978), and ^{13}C chemical shifts of thiazolium C(2) (Gallo & Sable, 1976).

Values of $pK_1 = 5.1$ and $pK_3 = 8.6$ in Scheme II were determined as described above. A value of $pK_4 = 6.1$ was calculated from a Hammett correlation for the ionization of 2-methyl-4-amino-5-R-pyrimidinium cations, which follow $pK_a = 6.52 - 5.39\sigma_m$ (Mizukami & Hirai, 1966), and $\sigma_m = -0.07$ for $R = CH_2NH_2$ (Exner, 1978). The value of $K_2 = 10^{-7.6}$ M was calculated from the relationship $K_2 = K_1K_3/K_4$.

The value of $\sigma_I = 0.070$ for the 2-methyl-4-amino-5-methylenepyrimidinyl group was calculated by substituting

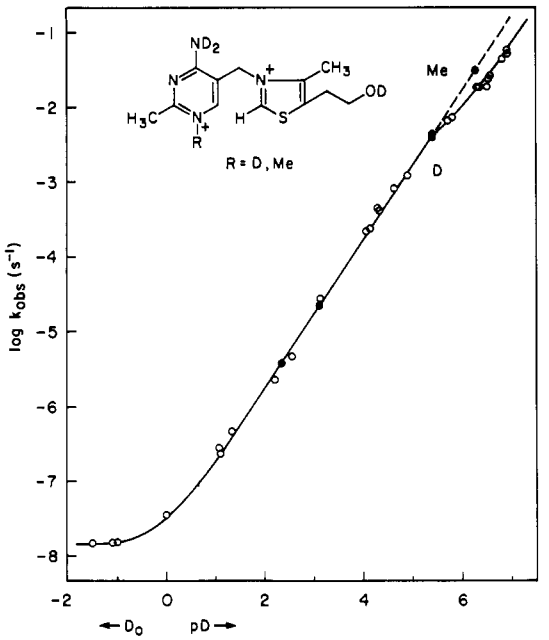


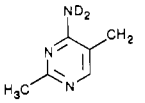
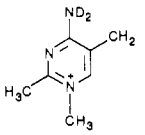
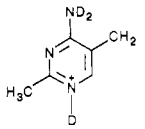
FIGURE 1: Dependence of the observed first-order rate constant for thiamin (○) and *N*(1′)-methylthiamin (●) C(2)H → C(2)D exchange on pD and the Hammett D_0 acidity function (Rochester, 1970) at 30 °C in D_2O. Above $D_0 = -0.7$ the ionic strength was maintained at 2.0 M (NaCl). The experiments were carried out in DCl below pD 3.5 and in 0.01 M acetate and phosphate buffers above pD 3.5; reactions with these buffers do not significantly affect the pD–log rate profile. The solid line drawn through the thiamin data is based on Scheme III using the rate law given in eq 4 with $k_{OD} = 8.4 \times 10^6$ M^{-1} s^{-1}, $k_{OD'} = 3.4 \times 10^6$ M^{-1} s^{-1}, $k_{D_2O} = 1.5 \times 10^{-8}$ s^{-1}, and $pK_a^{N1'} = 5.5$ (see text).

$pK_3 = 8.6$ into eq 3 to obtain σ_I for the 2-methyl-4-amino-pyrimidinyl group and then multiplying this σ_I value by 0.40 to correct for transmission of the inductive effect through the C(5)-methylene group (Wells, 1968). The value of $\sigma_I = 0.12$ for 2-methyl-4-amino-5-methylenepyrimidinium cation was calculated similarly by taking $pK_2 = 7.6$ for either H or Me at N(1). We estimate that these σ_I values are accurate to within ±0.01.

Results

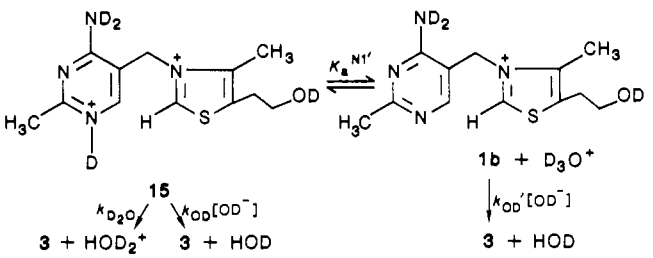
Pseudo-first-order and second-order rate constants for catalysis by D_2O and deuterioxide ion, respectively, of C(2)-proton exchange from thiamin and several 3-substituted-4-methylthiazolium ions at 30 °C and ionic strength 2.0 M (NaCl) are reported in Table I. Figure 1 shows the depen-

Table I: Rate Constants for Deuterioxide and D_2O-Catalyzed 3-R-4-methylthiazolium Ion C(2)-Proton Exchange[a]

	R	log k_{OD}[b] (M^{-1} s^{-1})	$10^9 k_{D_2O}$ (s^{-1})	pK_a[c] (D_2O)	pK_a[c] (OD^-)	σ_I[d]
4	Me	5.63	≤1.6	≥18.7	18.9	−0.05
5	Et	5.51				−0.05
6	Pr	5.58				−0.04
7	$CH_2CH_2COO^-$	5.63				−0.03[e]
8	$CH_2CH{=}CH_2$	5.99				0.01
9	CH_2COO^-	6.03				0.01
10	Bzl	6.33				0.04
11	4-NO_2-Bzl	6.78	11	17.8	17.8	0.092[f]
12	CH_2CO_2Et	6.94				0.13
13	CH_2CN	7.67	94	16.9	16.9	0.20[g]
1b		6.53			18.0	0.070[h]
14		6.88				0.12[h]
15		6.92	15	17.7	17.6	0.12[h]

[a] 30 °C and ionic strength 2.0 M (NaCl) in D_2O. [b] Concentration based, γ_{OD} = 0.85. [c] pK_a for the thiazolium C(2) proton in H_2O, calculated from rate constants for catalysis by D_2O and OD^- (see text). [d] Charton (1964). [e] σ_I was calculated from $\sigma_I = 0.217\sigma^* - 0.106$ and $\sigma^* = 0.35$ (Perrin et al., 1981). [f] σ_I was calculated from $\sigma_I = 0.23$ for R = 4-NO_2-Ph (Charton, 1981) and an attenuation factor of 0.40 for a methylene group (Wells, 1968). [g] Charton (1981). [h] This work.

Scheme III



dence on pD and the Hammett acidity function D_0 (Rochester, 1970) of the observed pseudo-first-order rate constants for C(2)-proton exchange of free thiamin (**1b**), N(1′)-protonated thiamin (**15**, see Table I), and *N*(1′)-methylthiamine (**14**). Similar data were obtained for other 3-R-4-methylthiazolium ions, with R = Me (**4**, see Table I), 4-NO_2-Bzl (**11**), and $NCCH_2$ (**13**). The D_0 acidity function in DCl/D_2O mixtures (10^{-4}–1.5 M) is identical with the H_0 acidity function in aqueous HCl for substituted aniline indicators (Högfeldt & Bigeleisen, 1960; Rochester, 1970) and is temperature independent (Paul & Long, 1957; Inoue, 1975). This pD–log rate profile is described by Scheme III and the rate law given in eq 4 for the formation of free or N(1′)-protonated thiamin

$$k_{obsd} = \frac{k_{OD}'[OD^-]K_a^{N1'}/a_{D^+} + k_{OD}[OD^-] + k_{D_2O}}{1 + K_a^{N1'}/a_{D^+}} \quad (4)$$

C(2)–D (**3**, Scheme I) using $k_{OD}' = 3.4 \times 10^6$ M^{-1} s^{-1} for free thiamin (**1b**) and data for N(1′)-protonated thiamin (**15**) with $k_{OD} = 8.4 \times 10^6$ M^{-1} s^{-1}, $k_{D_2O} = 1.5 \times 10^{-8}$ s^{-1}, and p$K_a^{N1'}$-(D_2O) = 5.5. The ratio $k_{OD}/k_{OD}' = 2.5$ agrees with the relative C(2)-proton exchange rates of 2.5:1 for *N*(1′)-methylthiamin (**15**) over free thiamin (**1b**) determined by an NMR line-broadening method at pH 8.2 in H_2O (Jordan & Mariam, 1978), which is approximately 1.5 pD units greater than the highest pD used in our experiments.

The pD–log rate profile for thiamin (Figure 1) indicates a change in the pathway for C(2)-proton exchange by the upward deviation in the profile at pD ≈ 0 from pD-independent D_2O to deuterioxide ion catalyzed exchange. The downward deviation in the profile near pD 5.5 reflects the decrease in exchange rate for deuterioxide ion catalyzed C(2)-proton exchange upon deprotonation of N(1′)-protonated thiamin (**15**), with p$K_a^{N1'}$ = 5.5 in D_2O, to form the less reactive free thiamin (**1b**). The absence of a downward deviation for *N*-(1′)-methylthiamine (**14**) (Figure 1) is consistent with this explanation.

The rate constants for D_2O-catalyzed C(2)-proton exchange are similar to upper limits on k_{H_2O} estimated for C(2) detritiation of several 3-substituted thiazolium salts, including N(1′)-protonated thiamine (**15**), in the range $k_{H_2O} \leq (0.03\text{–}1) \times 10^{-9}$ M^{-1} s^{-1} at 30 °C and ionic strength 1.0 M (NaCl) (Kemp & O'Brien, 1970). The deuterioxide ion catalyzed C(2)-proton exchange rates for free thiamin (**1b**), N(1′)-protonated thiamin (**15**), and *N*(1′)-methylthiamine (**14**) were found to be independent of the concentration of either thiamin or *N*(1′)-methylthiamin in the range 0.1–0.4 M, as observed by others (Gallo & Sable, 1976). Previously reported values of k_{OD} for the ionization of 3-R-4-methylthiazolium salts of 1.3×10^5 M^{-1} s^{-1} (25 °C, I = 1.0 M, concentration based) (Crosby & Lienhard, 1970) and 3.7×10^5 M^{-1} s^{-1} (32 °C, activity based) (Haake et al., 1970) for R = Me (**4**, see Table I), $k_{OD} = 1.5 \times 10^6$ M^{-1} s^{-1} (38 °C, activity based) (Haake et al., 1970) for R = Bzl (**10**), and $k_{OD} = 1.1 \times 10^7$ M^{-1} s^{-1} [30 °C, I = 1.8 M (NaCl), concentration based] (Gallo & Sable, 1976) for N(1′)-protonated thiamin (**15**) are in reasonable agreement with the rate constants reported here (Table I).

Table I also contains σ_I values for the 3-substituents (Charton, 1964, 1981). Values of σ_I for the free and protonated 2-methyl-4-amino-5-methylenepyrimidinyl group were calculated as described under Experimental Procedures.

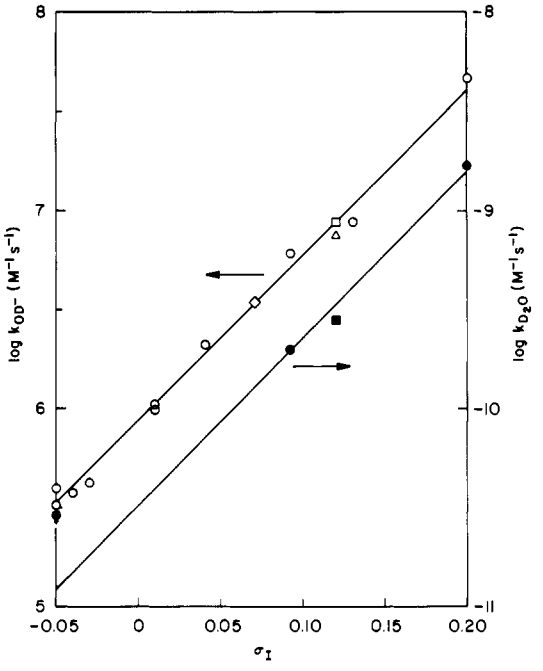


FIGURE 2: Hammett correlation with σ_I for C(2)H → C(2)D exchange of 3-R-4-methylthiazolium ions catalyzed by deuterioxide ion (open symbols) and D_2O (solid symbols) at 30 °C and I = 2.0 M (NaCl). The second-order rate constant for D_2O-catalyzed exchange was calculated by dividing the observed pseudo-first-order rate constant by 55.1 M, the concentration of D_2O at 30 °C (Millero et al., 1971). The squares are for thiamin protonated at the N(1′) position, the triangle is for *N*(1′)-methylthiamin, and the diamond is for free thiamin. The upper limit for catalysis by D_2O when R = Me is indicated by the solid circle on the left.

DISCUSSION

Inductive Effects. The second-order rate constants for reactions of deuterioxide ion and D_2O with 3-R-4-methylthiazolium ions, including thiamin, follow Hammett correlations with the inductive substituent constant σ_I for R with a slope of ρ_I = 8.4 ± 0.2 for both catalysts, as shown in Figure 2. A dependence of the C(2)-proton exchange rate on an electron-withdrawing inductive effect by substituents on the nitrogen atom of the thiazolium ring has been noted by others (Breslow, 1958; Yount & Metzler, 1959; Gallo & Sable, 1974, 1976). The value of ρ_I = 8.4 for thiazolium C(2)-proton exchange is similar to ρ_I = 8.0 for hydroxide ion catalyzed detritiation of substituted acetylenes in aqueous solution (Kresge & Powell, 1986). The acidities of "normal" acids, such as substituted alcohols and aliphatic ammonium ions, have also been satisfactorily correlated with a value of ρ_I = −8.4 (Fox & Jencks, 1974). The electron pair of acetylide ions resides in an sp hybridized orbital, which is orthogonal to the acetylenic π-system (Hopkinson, 1978), and is highly localized on the terminal carbon atom. These similar ρ_I values for acetylide ions and thiazolium C(2) ylides are consistent with the development of a large amount of negative charge at the ionizing carbon in the transition state for the thiazolium C(2)-proton transfer and indicate that thiazolium C(2) ylides are similar to the electronegative atoms of normal bases, where the basic electron pair is highly localized on single atoms (Eigen, 1964).

The ρ_I value of 8.4 for both thiazolium ions and normal acids supports the conclusion that there is little, if any, reasonance that decreases the positive charge on N(3) in the C(2) ylide; N(3) is closer to the substituent, and a decrease in its charge would give a larger value of ρ_I. Thus, the identical ρ_I values for normal acids and thiazolium C(2) ylides provide no evidence that stabilization of the C(2) ylide by a resonance contribution from a carbene-like structure (**2b**, Scheme I) is important. The formation of a rearranged dimer for thiamin, 3-benzylthiazolium, and 3-benzylbenzothiazolium salts was suggested to occur through a mechanism involving carbene dimerization followed by a 1,3-sigmatropic rearrangement of the benzyl (or the aminopyrimidinyl) group (Doughty & Risinger, 1987); however, an ionic mechanism involving C-(2)-ylide addition to the thiazolium ring at C(2) followed by deprotonation and rearrangement was not ruled out.

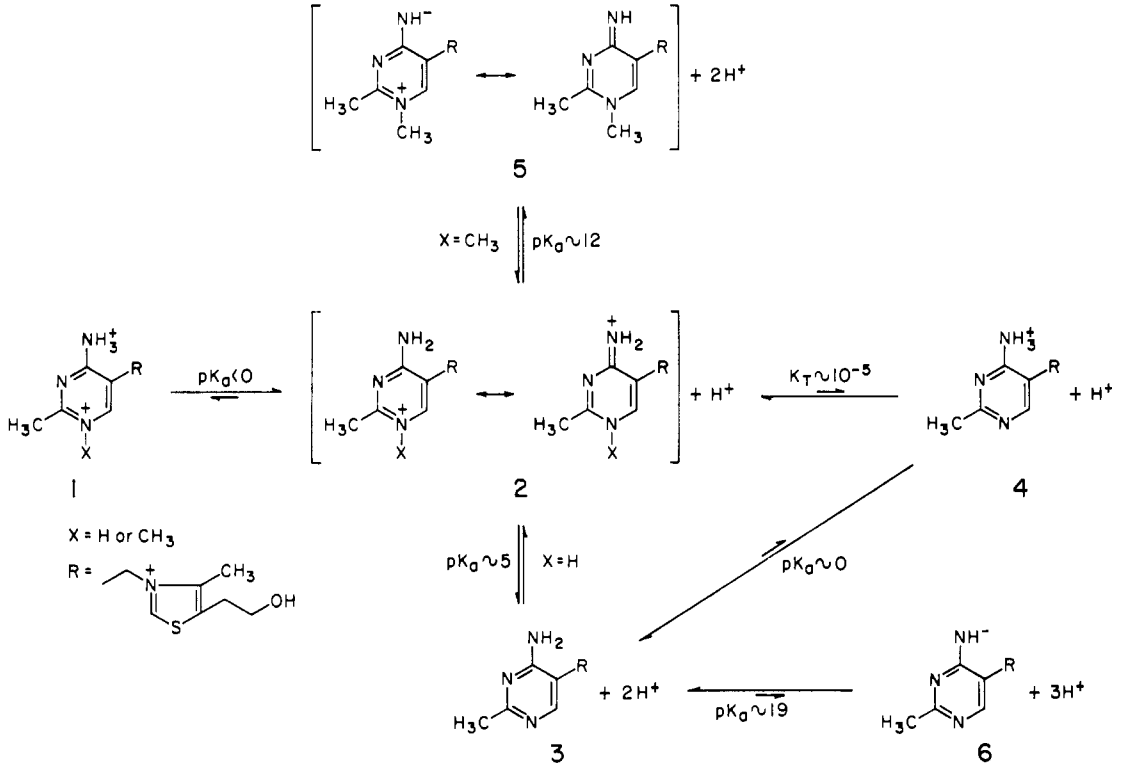
Intramolecular Proton Transfer. There is no positive deviation in Figure 2 of the rate constants for catalysis by either D_2O or OD^- for 3-(carboxyethyl)-4-methylthiazolium ion (**7**, see Table I), free thiamin (**1b**), N(1′)-protonated thiamin (**15**), or *N*(1′)-methylthiamin (**14**), which contain potential intramolecular general base catalysts. The pD–log rate profile for thiamin (Figure 1) is described by Scheme III and the rate law given in eq 4 without a term for inter- or intramolecular general base catalysis of C(2)-proton transfer. This shows that the aminopyrimidinyl group does not provide significant intramolecular base catalysis of C(2)-proton removal in the coenzyme. The observation that the rate for C(2)-proton exchange is independent of thiamin concentration indicates that the aminopyrimidinyl group also does not provide significant intermolecular general base catalysis of C(2)-proton removal.

Possible resonance forms and equilibria to be considered when the role of the aminopyrimidinyl group in the C(2)-proton exchange reaction is discussed are shown in Scheme IV. In the pH range 0–9 only $pK_a \approx 5$ for the protonated N(1′) position is observed (**2** ⇄ **3**, Scheme IV) (Suchy et al., 1972; Gallo & Sable, 1975; Jordon & Mariam, 1978). Other equilibria [**1** ⇄ **2**, **2** ⇄ **4**, **3** ⇄ **4** (Stewart & Harris, 1978; Zoltewicz et al., 1978), and **3** ⇄ **6** (Jordon et al., 1982) in Scheme IV] are too unfavorable to be significant in aqueous solution in this pH range. Protonation or alkylation at N(1′) to form the pyrimidinium cation makes the exocyclic 4′-amino group a weak acid. Jordon has determined the pK_a for **2** ⇄ **5** (Scheme IV) to be 12.7 for X = Me and R = CH_2OCH_3 by potentiometric titration (Jordon & Mariam, 1978) and 7.5 for X = H and R = CH_2OCH_3 by stopped-flow kinetics (Jordan et al., 1982). Consequently, the various equilibria strongly favor structures **3** ⇄ **2** ⇄ **5** (Scheme IV) in the physiologically relevant pH range, and the only potential general base catalysts are the exocyclic 4′-imino group and the heterocyclic N(1′) position.

Several workers have proposed that an enzyme could activate the exocyclic 4′-amino group catalytically by attachment of a positive charge at N(1′) by protonation or attachment of the Mg^{2+} ion known to be required for catalysis (Krampitz, 1969) or by interaction with a positively charged amino acid side chain from the enzyme (Schellenberger, 1967; Jordan & Mariam, 1978). The N(1′) position has been implicated as a site for binding of a divalent metal ion on the basis of the pH dependence of the width of the ^1H NMR signal for C(6′)-H of TPP in the presence of a divalent metal ion (Gallo et al., 1972; Gallo & Sable, 1975). Jordan demonstrated that the amino group is converted to a weak acid in *N*(1′)-methylthiamin (**14**) that can ionize to a strong conjugate base (**2** ⇄ **5**, Scheme IV) and proposed that this exocyclic 4′-imino group might participate in intramolecular general base catalysis in proton transfers involving thiamin (Jordan & Mariam, 1978; Jordan et al., 1982).

A covalent bond at the N(1′) position to an alkyl group or a proton is expected to have a larger effect than electrophilic catalysis by complexation at N(1′) with a metal ion or a

Scheme IV



positively charged amino acid side chain. For example, the effect of an alkyl group or a proton that forms an additional covalent bond to a phosphoryl oxygen atom of a phosphate monoester on the hydrolysis rate and transition-state structure is much larger (Kirby & Vargolis, 1968; Kahn & Kirby, 1970; Kirby & Younas, 1970) than the effect of complexation with metal ions (Herschlag & Jencks, 1987). The results for *N*-(1′)-methylthiamin (**14**) show that attachment of a methyl group to the N(1′) position has no effect, other than inductive, on the nonenzymic C(2)-proton exchange rate (Figure 2). Therefore, these mechanisms to activate the exocyclic 4′-amino group do not provide sufficient activation to allow this group to function as an intramolecular general base catalyst for C(2)-proton exchange.

Schellenberger and Petzold and their co-workers have proposed that the exocyclic 4′-amino group functions as an intramolecular general base catalyst for C(2)-proton abstraction via **3** ⇄ **4** (Scheme IV) in both the enzymic and nonenzymic exchange reactions on the basis of steric considerations (Schellenberger, 1967) and on ^{1}H NMR determination of the C(2)-proton exchange rates of TPP and several TPP derivatives (Petzold, 1976; Petzold et al., 1982). This mechanism is unlikely in aqueous solution because of the low pK_a value of about −0.3 for the protonated 4′-amino group (Gallo & Sable, 1976; Zoltewicz et al., 1978), and this mechanism is not indicated in Figure 2, where the second-order rate constants for C(2)-proton exchange of free thiamin (**1b**) and *N*(1′)-methylthiamin are on the same line. The exocyclic 4′-amino group of thiamin does participate in an intramolecular cyclization with the thiazolium ring in basic solution to form a neutral tricyclic species (Hopmann, 1982; Doughty & Risinger, 1985).

Why is intramolecular proton transfer between thiazolium C(2) and the aminopyrimidinyl group not observed? Whether the intramolecular pathway can compete significantly with the pathways involving external bases (OD^- and D_2O) depends on the Brønsted β value for general base catalysis of proton transfer, the strength and concentration of the base catalyst, and geometrical constraints on the intramolecular base catalyst (Bernasconi et al., 1982).

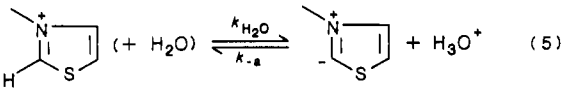
Abstraction of the C(2) proton from thiazolium ions follows almost completely normal "Eigen curves" (Eigen, 1964), with Brønsted β values ≥0.95 for catalysis by buffer bases (Washabaugh and Jencks, unpublished results). Buffer catalysis is detectable because there is a small negative deviation from this correlation for deuterioxide ion. A Brønsted β value of 1.0 does not exclude the intramolecular pathway, it just makes it somewhat less favored relative to the specific base catalyzed pathway. Intramolecular general base catalysis would have been seen if the "effective molarity"[2] of the exocyclic 4′-imino group were large. Typical effective molarities (Kirby, 1980) for intramolecular proton transfer between carbon and oxygen or nitrogen bases are in the range of ≈0.1–1 M when the carbon skeleton is flexible (Bell, 1973; Bernasconi & Murray, 1986) or up to ≈50 M when the carbon skeleton is more rigid (Harper & Bender, 1965). An upper limit for the effective molarity of the exocyclic 4′-imino group of *N*(1′)-methylthiamin of ≤0.4 M can be estimated for intramolecular general base catalysis of C(2)-proton exchange.[3]

pK_a Values of 3-R-4-methylthiazolium Ions. Values of pK_a for C(2)–H in several 3-substituted-4-methylthiazolium ions,

[2] The effective molarity is equal to the rate constant for the intramolecular reaction divided by the rate constant for an intermolecular proton transfer to an external acid or base of the same pK_a as that of the internal acid or base (Page, 1973; Kirby, 1980).

[3] The upper limit for the effective molarity (EM) of the exocyclic 4′-imino group (pK_a = 12.7) for intramolecular general base catalysis of C(2)-proton exchange from *N*(1′)-methylthiamin was calculated from EM = k_{intra} (s^{-1})/k_{inter} (M^{-1} s^{-1}) and an error of ±5% in k_{obsd} at pD 6.55, which is the highest pD examined for this compound. The value for k_{intra} was calculated from $k_{intra} = 0.05k_{OD}[OD^-]/$(fraction base as anion), where $k_{OD} = 7.6 \times 10^6$ M^{-1} s^{-1} for *N*(1′)-methylthiamin (**14**, see Table I) and $[OD^-] = 8.33 \times 10^{-9}$ M ($\gamma_{OD} = 0.85$). The value for k_{inter} of 1.26×10^4 M^{-1} s^{-1} for a general base with pK_a = 12.7 is from a Brønsted plot of slope β = 1.0 for general base catalysis of C(2)-L exchange from N(1′)-protonated thiamin in D_2O at 30 °C and I = 2.0 M (NaCl) (Washabaugh and Jencks, unpublished results).

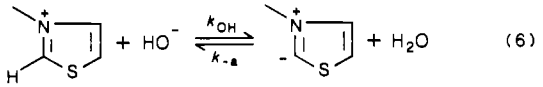
including N(1′)-protonated thiamin, were found to be in the range pK_a = 17–19 in H_2O (Table I). These equilibrium constants in H_2O were obtained according to eq 5. The value



(5)

of k_{H_2O} was calculated by multiplying the observed pseudo-first-order rate constant for the pD-independent, buffer-independent C(2)-proton exchange reaction with D_2O by 2.6, to correct for the secondary solvent deuterium isotope effect on the rate constant of k_{H_2O}/k_{D_2O} = 2.6. A value of k_{-a} = 2 × 10^{10} M^{-1} s^{-1} was assumed for the diffusion-controlled reaction in the reverse direction. Values for k_{-a} in the range (1–4) × 10^{10} M^{-1} s^{-1} have been reported for protonation of amines by H_3O^+ (Eigen, 1964), and a rate constant of 4 × 10^{10} M^{-1} s^{-1} has been measured for the diffusion-controlled protonation of CN^- by H_3O^+, which is a localized carbanion similar to the thiazolium C(2) ylide (Bednar & Jencks, 1985). This uncertainty in k_{-a} results in an uncertainty of ±0.3 in the calculated pK_a values. The assumption of diffusion-controlled reprotonation of thiazolium C(2) ylides by H_3O^+ is supported by the Brønsted β values ≥0.95 for catalysis of thiazolium C(2)-proton exchange by buffer bases, which indicate that the reverse protonation reaction has a Brønsted α value of ≤0.05 and that protonation is diffusion-controlled with strong acids (Washabaugh and Jencks, unpublished results). The solvent isotope effect of k_{H_2O}/k_{D_2O} = 2.6 was calculated from a fractionation factor of $l^2 = (0.69)^2$ for HOD_2^+ and a solvent isotope effect of 1.22 (Millero et al., 1971) for diffusional separation of HOD_2^+ from the thiazolium C(2) ylide (Fong & Grunwald, 1969; Bednar & Jencks, 1985).[4]

The C(2)–H pK_a values of 3-R-4-methylthiazolium ions obtained from exchange catalyzed by D_2O are confirmed by values calculated from the observed rate constants for catalysis by OD^-, pK_a = 15.57 for H_2O at 30 °C, and the equation pK_a = 15.57 − log (k_{OH}/k_{-a}) according to eq 6 (Table I). The



(6)

value of k_{OH} was calculated by multiplying k_{OD} by 3.3 = 8/2.4 to correct for an 8-fold negative deviation of k_{OD} from the Brønsted plot of slope 1.0 that was observed for base-catalyzed proton exchange from 3,4-dimethylthiazolium ion under conditions in which diffusion-controlled separation of the products is rate-limiting (Washabaugh and Jencks, unpublished results) and a secondary isotope effect of k_{OD}/k_{OH} = 2.4 (Gold & Grist, 1972).[5] A value of k_{-a} = 3 × 10^9 M^{-1} s^{-1} was assumed for the diffusion-controlled protonation of the carbanion by water (Keeffe et al., 1986).[6] These rate constants give pK_a = 18.0 for free thiamin (**1b**) and pK_a = 17.6 for N(1′)-protonated thiamin (**15**, see Table I) [30 °C and ionic strength 2.0 M (NaCl)]. This pK_a value of 17.6 for N(1′)-protonated thiamin agrees well with pK_a = 17.7 obtained from the water reaction. This procedure allows HO^- to be treated as a buffer base of pK_a = 15.57 with no deviation from the Brønsted plot. Previous estimates of thiazolium C(2)–H pK_a values calculated from k_{OD} did not correct for this negative deviation and assumed that there was no activation energy for k_{-a} (McNelis, 1960) or values for k_{-a} of 1.8 × 10^3 M^{-1} s^{-1} (Haake et al., 1969), 10^{10} M^{-1} s^{-1} (Crosby & Lienhard, 1970), and 10^9 M^{-1} s^{-1} (Kluger, 1987). The reason for the negative deviation of deuterioxide ion from the Brønsted plot will be discussed elsewhere.

These C(2)–H pK_a values for 3-substituted thiazolium ions, including free and N(1′)-protonated thiamin, are much higher than the previous estimates of 12.7 for free thiamin (Hopmann & Brugnoni, 1973) and 14 for 3,4-dimethylthiazolium ion (Haake et al., 1969) but are in the same range as estimates by several other investigators for 3,4-dimethylthiazolium ion of 20 (McNelis, 1960) and 19.2 (Crosby & Lienhard, 1970), for 3-benzyl-4,5-dimethylthiazolium ion of 18–20 (Kemp & O'Brien, 1970), and for N(1′)-protonated thiamin of 17–19 (Kemp & O'Brien, 1970) and 19 (Kluger, 1987). The pK_a estimates by Kemp and O'Brien are based on upper limits on C(2) detritiation rates for catalysis by H_2O assuming a value of k_{-a} = 10^{10}–10^{11} M^{-1} s^{-1} (Kemp & O'Brien, 1970). It appears likely that the pK_a of 12.7 is actually pK_{R^+} for the formation of a neutral tetrahedral intermediate by nucleophilic attack at C(2) in the thiazolium cation ring by hydroxide ion, which is the first step in hydroxide-catalyzed hydrolysis of the thiazolium ring (Bunting, 1979).

The pK_a range of 17–19 for thiazolium C(2)–H cations indicates that their proton-transfer reactions are thermodynamically unfavorable in aqueous solution. The conclusion that protonation of the C(2) ylide is diffusion-controlled means that proton loss from these carbon acids involves rate-limiting diffusional separation of the proton-transfer products. This is relevant to the physiological role of thiamin because it means that C(2)-proton removal occurs at the maximum possible rate for a given equilibrium constant.

Implications for Enzyme-Catalyzed Reactions. Aldol-type addition reactions between thiamin and carbonyl compounds are catalyzed by several thiamin-dependent enzymes (Krampitz, 1969). It is not known whether the thiazolium C(2) ylide exists as a discrete carbanion intermediate on these enzymes. A pK_a value of ≤14 is required for enzyme-bound thiamin C(2)–H if the thiazolium ylide exists as an intermediate in a stepwise enzymic addition reaction (Crosby & Lienhard, 1970; Kemp & O'Brien, 1970). Therefore, these enzymes either change the relative thermodynamic stabilities of thiamin and its ylide or they provide a one-step, concerted pathway that avoids an unstable carbanion intermediate (Thibblin & Jencks, 1979). The pK_a values for thiazolium C(2) ylides in the range 17–19 that are reported here show that the carbanion is quite unstable in aqueous solution. However, rate-limiting diffusion-controlled protonation and deprotonation in this system mean that there is internal return in C(2)-proton exchange and proves that thiazolium ylides have a significant lifetime in aqueous solution.

The mechanism of carbanion addition to carbonyl compounds in aqueous solution has been investigated for substituted cyanohydrin formation (where CN^- is the nucleophile) (Ching & Kallen, 1978), which is similar to the aldol-type addition reaction between thiamin and carbonyl compounds. Cyanide addition to aldehydes exhibits only specific base catalysis, consistent with a stepwise mechanism. Because the thiamin C(2) ylide has a significant lifetime in aqueous solution and is much more basic than CN^-, the aldol-type addition

[4] This secondary solvent deuterium isotope effect was observed for 3-(cyanomethyl)-4-methylthiazolium ion, which gives k_{H_2O}/k_{D_2O} = 2.8 ± 0.2 for transfer of C(2)–H to water. The reaction was followed by ^3H incorporation into the C(2)-H substrate (Washabaugh and Jencks, unpublished results).

[5] This secondary solvent isotope effect was confirmed for 3,4-dimethylthiazolium ion, which gives k_{OD}/k_{OH} = 2.4 ± 0.2 for transfer of C(2)–^3H to lyoxide ion (Washabaugh and Jencks, unpublished results).

[6] These workers measured the rate constant directly for the diffusion-controlled reaction of acetophenone enol with bromine in aqueous hydrobromic acid at 25 °C and I = 0.10 M (NaBr).

reaction of thiamin C(2) ylide must follow either a stepwise pathway or a nonenforced concerted pathway (Palmer & Jencks, 1980). The stepwise pathway must exist, but it might be bypassed by a nonenforced concerted pathway because of the relative instability of the carbanion. The mechanism could be concerted, in principle, but this is unlikely; steric requirements would require frontside electrophilic attack by the carbonyl group on the C(2)–H bond of thiamin.

It is possible that thiamin-dependent enzymes could substantially decrease the pK_a value of enzyme-bound TPP C(2)–H. With TPP and other positively charged acids ionizing according to eq 7, where there is a change in the number of

$$A^+–H + B^- \rightleftharpoons A^{\pm} + H–B \qquad (7)$$

ions, the equilibrium is very sensitive to the dielectric constant of the medium; the pK_a decreases markedly (the acid becomes stronger) in solvents of low dielectric constant. Spectroscopic studies suggest that the TPP binding sites of yeast pyruvate decarboxylase (Wittorf & Gubler, 1970), yeast transketolase (Kochetov & Usmanov, 1970), and *Escherichia coli* pyruvate oxidase (O'Brien & Gennis, 1980) are nonpolar. The relatively weak binding of TPP (Gutowski & Lienhard, 1976) and the pyruvate–TPP adduct, α-lactylthiamin diphosphate (Kluger & Smyth, 1981), to pyruvate decarboxylase might be because a large amount of the intrinsic binding energy, provided by the pyrophosphate and aminopyrimidinyl group as well as the thiazolium ring, is used to compensate for the loss of solvation when the positively charged thiazolium ring and the carboxylate group of α-lactylthiamin diphosphate are brought into the nonpolar active site (Crosby et al., 1970; Jencks, 1975).[7] On the basis of the pH dependence of inactivation of yeast transketolase by a carbodiimide, an essential carboxylate group with a pK_a of about 6.5 was identified and proposed to function as a general base catalyst for thiamin C(2)-proton transfer (Kuimov et al., 1985). The lowering of pK_a values by enzymes has precedent: (1) In acetoacetate decarboxylase the pK_a of the protonated ϵ-amino group of an active-site lysine is decreased from the value for free lysine by about 4.7 pK units, to a value of 6.0 (Schmidt & Westheimer, 1971); (2) the pK_a of the hemiketal hydroxyl in the complex between α-chymotrypsin and the inhibitor *N*-acetyl-L-leucyl-L-phenylalanyl trifluoromethyl ketone is approximately 4.9, which is about 4.2 pK units lower than the pK_a of model hemiketals (Liang & Abeles, 1987).

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