

## A Comparison of the Electrophilic Reactivities of $\text{Zn}^{2+}$ and Acetic Acid as Catalysts of Enolization: Imperatives for Enzymatic Catalysis of Proton Transfer at Carbon

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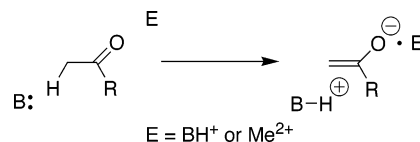
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**Abstract:** The deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone and the  $\alpha\text{-CH}_3$  groups of acetone in the presence of acetate buffer and zinc chloride in  $\text{D}_2\text{O}$  at 25 °C was followed by monitoring the incorporation of deuterium by  $^1\text{H}$  NMR spectroscopy, and the rate laws for catalysis of these reactions by acetate anion and zinc dication were evaluated. Relative to solvent water at a common standard state of 1 M,  $\text{Zn}^{2+}$  provides 6.3 and 4.4 kcal/mol stabilizations, respectively, of the transition states for deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  and  $\alpha\text{-CH}_3$  groups of hydroxyacetone by acetate anion, and a smaller 3.3 kcal/mol stabilization of the transition state for deprotonation of the  $\alpha\text{-CH}_3$  group of acetone. There is only a 1.4 kcal/mol smaller stabilization of the transition state for the acetate-ion-promoted deprotonation of acetone by the Brønsted acid acetic acid than by  $\text{Zn}^{2+}$ , which shows that, in the absence of a chelate effect, there is no large advantage to the use of a metal dication rather than a Brønsted acid to stabilize the transition state for deprotonation of  $\alpha$ -carbonyl carbon.

Proton transfer from carbon acids to Brønsted bases is a simple chemical reaction that is a vital step of many complex biological processes. Proton transfer is strongly catalyzed by enzymes; however, as for many other simple reactions, there is no consensus about the origin of the enzymatic rate acceleration.<sup>1–7</sup> Deprotonation of  $\alpha$ -carbonyl carbon gives a delocalized “carbanion” where the negative charge lies mainly at the enolate oxygen that is derived from the neutral carbonyl oxygen at the substrate.<sup>8</sup> This negative charge provides a target for stabilization by interaction with Brønsted acids or metal ion electrophiles in catalyzed reactions (Scheme 1).

There is no obvious pattern in the metal ion requirements of enzymes that catalyze proton transfer at carbon. For example, mannose 6-phosphate isomerase<sup>9</sup> is a metalloenzyme while triosephosphate isomerase<sup>10</sup> and glucose 6-phosphate isomerase<sup>11</sup> is not. The observation that enzyme catalysts may use either

Scheme 1



metal cations or Brønsted acids to stabilize negative charge that develops at the enolate oxygen shows that both types of catalysis are viable, but the imperatives for the observation of catalysis by one mechanism rather than another have not been well defined. As a result of their high positive charge density, metal dications might be predicted to be superior to Brønsted acids as electrophilic catalysts of deprotonation of carbon.<sup>6</sup> However, Brønsted acid catalysis will be favored when there is a favorable thermodynamic driving force for proton transfer to the enolate oxygen that is partly expressed in the transition state.<sup>12</sup>

The rate acceleration for an enzyme-catalyzed reaction can be calculated using the rate constant for the corresponding uncatalyzed reaction in water as a point of reference.<sup>13</sup> The maximum rate acceleration for a given transition state stabilization will be observed when enzymatic catalysis occurs by the same mechanism as that observed for the reaction in water.<sup>14</sup> This provides an important motivation for characterizing model reactions in water of enzyme-catalyzed reactions.<sup>14,15</sup> There have

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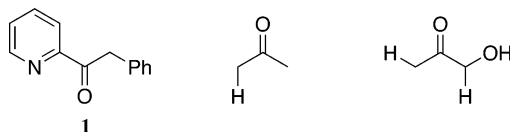
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been several studies of metal ion catalysis of the deprotonation of relatively acidic carbon acids such as **1**, which are activated



for proton transfer by a second substituent (e.g., a phenyl group) and contain a basic site for chelation the metal ion.<sup>16–19</sup> By comparison, there are only limited data for metal ion catalysis of the slower deprotonation of weakly acidic α-carbonyl carbon acids that are relevant models for enzyme-catalyzed deprotonation of keto sugars.<sup>14,20</sup> The lack of model studies of metal ion catalysis of proton transfer from simple carbon acids has impeded discussions of the relative advantages of Brønsted acid and metal ion catalysis by enzymes.<sup>1,6</sup>

Both hydroxyacetone and acetone are ideal substrates for studies of metal ion catalysis of the deprotonation of ketones: (1) There are literature data<sup>21,22</sup> for catalysis of the deprotonation of acetone by Brønsted acids that may be used in combination with new data for metal ion catalysis to obtain the relative stabilization of the transition state for proton transfer by interaction with these different electrophiles. (2) A comparison of metal ion catalysis of deprotonation of acetone and hydroxyacetone will provide a simple estimate of the contribution to catalysis available from chelation of the metal ion by the substrate. (3) Additional insight into organic reactivity and reaction mechanism can be obtained from a comparison of kinetic data for deprotonation of the α-CH<sub>3</sub> and α-CH<sub>2</sub>OH groups of hydroxyacetone.<sup>23</sup>

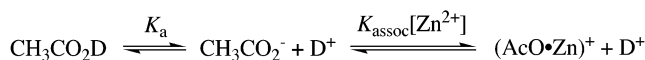
## Experimental Section

**Materials.** Hydroxyacetone (Fluka), acetone (Mallinckrodt), ZnCl<sub>2</sub> (Baker), potassium acetate (Aldrich), and all other inorganic salts were reagent grade or better and were used without further purification. Deuterium oxide (99.9% D) and deuterium chloride (35% w/w, 99.5% D) were purchased from Cambridge Isotope Laboratories. Potassium deuterioxide (40wt %, 98+% D) was purchased from Aldrich.

**Preparation of Acetate Buffers Containing Zn<sup>2+</sup>.** Solution pD was determined at 25 °C using an Orion Model 720A pH meter equipped with a Radiometer GK2321C combination electrode that was standardized at pH 7.00 and 4.00. Values of pD were obtained by adding 0.4 to the observed reading of the pH meter.<sup>24</sup>

The addition of Zn<sup>2+</sup> to acetate buffers leads to the formation of a complex between Zn<sup>2+</sup> and acetate anion that results in a decrease in the ratio of the concentrations of the basic and acidic forms of the buffer, [AcO<sup>-</sup>]/[AcOD], and a decrease in solution pD (Scheme 2). An apparent value of pK<sub>a</sub> = 5.06 for acetic acid in D<sub>2</sub>O at 25 °C and I = 1.0 (KCl) was determined from the measured pD of acetate buffers prepared in the absence of Zn<sup>2+</sup>. A value of K<sub>assoc</sub> = 7.0 M<sup>-1</sup> for formation of the (AcO·Zn)<sup>+</sup> complex between acetate anion and Zn<sup>2+</sup>

## Scheme 2



in D<sub>2</sub>O at 25 °C and I = 1.0 (KCl) was determined by assuming that the concentration of this complex is equal to the decrease in [AcO<sup>-</sup>] that can be calculated from the observed decrease in the pD of acetate buffers upon addition of known amounts of Zn<sup>2+</sup>, using pK<sub>a</sub> = 5.06 for acetic acid in D<sub>2</sub>O.

Solutions of acetate buffer in D<sub>2</sub>O at pD 5.70 and I = 1.0 (KCl) containing Zn<sup>2+</sup> were prepared by mixing KOAc, KCl, and ZnCl<sub>2</sub> followed by the addition of DCl to give a final pD of 5.70. The required amount of DCl was calculated from pK<sub>a</sub> = 5.06 for acetic acid and K<sub>assoc</sub> = 7.0 M<sup>-1</sup> for formation of the (AcO·Zn)<sup>+</sup> complex in D<sub>2</sub>O (see above). Small adjustments of the initial pD of these solutions (<0.10 unit) were made, as needed, to bring the final pD to 5.70.

**<sup>1</sup>H NMR Analyses.** <sup>1</sup>H NMR spectra at 500 MHz were recorded in D<sub>2</sub>O at 25 °C using a Varian Unity Inova 500 spectrometer. Values of T<sub>1</sub> = 6 and 7 s, respectively, were determined for the α-CH<sub>2</sub>OD and α-CH<sub>3</sub> protons of hydroxyacetone using a solution of 5 mM substrate in D<sub>2</sub>O at I = 1.0 M (KCl). These are similar to T<sub>1</sub> = 6 s reported for the α-CH<sub>3</sub> protons of acetone determined in chloroform.<sup>25</sup> Spectra were recorded with a sweep width of 6000 Hz, a 90° pulse angle, and an acquisition time of 6 s. The relaxation delay between pulses was at least 10-fold greater than the longest T<sub>1</sub> for the protons of interest. Chemical shifts are reported relative to HOD at 4.67 ppm. Baselines were subjected to a first-order drift correction before determination of integrated peak areas.

Small signals of around 1% the area of those for the methyl and methylene protons of the keto form of hydroxyacetone were observed in the region expected for the signals of the methyl and methylene protons of hydroxyacetone hydrate.

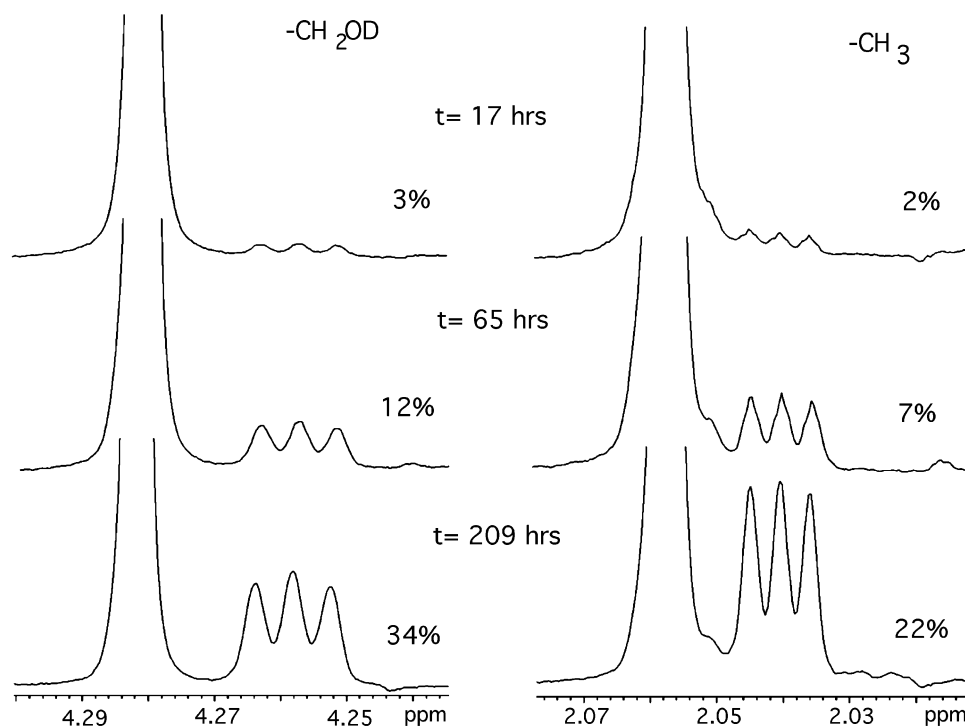
**Deuterium Exchange Reactions.** All reactions were carried out at pD 5.70 in D<sub>2</sub>O at 25 °C and I = 1.0 (KCl). Reactions were initiated by the addition of a small volume of a solution of acetone or hydroxyacetone in D<sub>2</sub>O to the reaction mixture to give a final substrate concentration of 15 mM.

The exchange for deuterium of the α-CH<sub>3</sub> protons of acetone and of hydroxyacetone was followed by <sup>1</sup>H NMR by monitoring the disappearance of the singlets at 2.202 and 2.058 ppm, respectively, due to the α-CH<sub>3</sub> group of the substrate and the appearance of triplets (J<sub>HD</sub> = 2.5 Hz) shifted upfield by 0.016 and 0.018 ppm, respectively, due to the α-CH<sub>2</sub>D groups of the products. The exchange for deuterium of the α-CH<sub>2</sub>OD protons of hydroxyacetone was followed by <sup>1</sup>H NMR by monitoring the disappearance of the singlet at 4.282 ppm due to the α-CH<sub>2</sub>OD group of the substrate and the appearance of the triplet (J<sub>HD</sub> = 2.5 Hz) shifted upfield by 0.023 ppm due to the α-CHDOD group of the product. These deuterium isotope effects on <sup>1</sup>H chemical shifts and H–D coupling constants are similar to those for related carbon acids that were reported in our earlier work.<sup>14,25–31</sup>

Values of R, which is a measure of the progress of the deuterium exchange reaction,<sup>32,33</sup> were calculated according to eq 1 for the reaction of α-CH<sub>3</sub> groups or eq 2 for the reaction of the α-CH<sub>2</sub>OD group of hydroxyacetone. In these equations, A<sub>CH<sub>3</sub></sub> and A<sub>CH<sub>2</sub></sub> are the integrated areas of the singlets due to the α-CH<sub>3</sub> and α-CH<sub>2</sub>OD groups of the

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**Figure 1.** Representative partial  $^1\text{H}$  NMR spectra of hydroxyacetone obtained during deuterium exchange in the presence of 0.34 M acetate buffer and 0.12 M added  $\text{Zn}^{2+}$  at pD 5.70 in  $\text{D}_2\text{O}$  at 25  $^\circ\text{C}$  and  $I = 1.0$  (KCl). The exchange for deuterium of the first proton of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups leads to disappearance of the singlets at 2.058 and 4.282 ppm, respectively, and the appearance of upfield-shifted triplets ( $J_{\text{HD}} = 2.5$  Hz) at 2.040 and 4.259 ppm. The extent of exchange of the first proton of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups at various reaction times is indicated at the right of each spectrum.

reactant and  $A_{\text{CH}_2\text{D}}$  and  $A_{\text{CHD}}$  are the integrated areas of the triplets due to the  $\alpha\text{-CH}_2\text{D}$  and  $\alpha\text{-CHDOD}$  groups of the product. At early reaction times it was sometimes possible to obtain baseline separation for only the most upfield of the three peaks of the triplet due to the  $\alpha\text{-CH}_2\text{D}$  group of the product. In these cases, the area of the triplet ( $A_{\text{CH}_2\text{D}}$ ) was calculated by multiplying the integrated area of the most upfield peak by three.<sup>26</sup> The area of the singlet due to the  $\alpha\text{-CH}_3$  group of the substrate ( $A_{\text{CH}_3}$ ) was then calculated as the difference between the total integrated area of all the signals due to the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{D}$  groups and that calculated for the triplet.

$$R = \frac{A_{\text{CH}_3}}{A_{\text{CH}_3} + \frac{A_{\text{CH}_2\text{D}}}{2}} \quad (1)$$

$$R = \frac{A_{\text{CH}_2}}{A_{\text{CH}_2} + A_{\text{CHD}}} \quad (2)$$

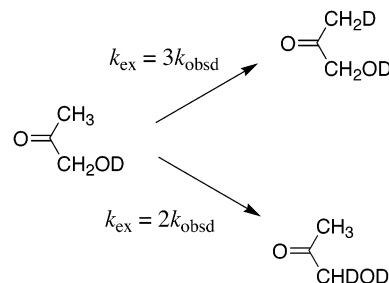
The reactions of the  $\alpha\text{-CH}_3$  groups of acetone and hydroxyacetone were followed during exchange for deuterium of up to 10% and 30%, respectively, of the first proton. The reaction of the  $\alpha\text{-CH}_2\text{OD}$  group of hydroxyacetone was followed during exchange for deuterium of up to 60% of the first proton. Semilogarithmic plots of reaction progress,  $R$ , against time were linear with negative slopes equal to  $k_{\text{obsd}}$  ( $\text{s}^{-1}$ , eq 3), where  $k_{\text{obsd}}$  is the rate constant for exchange of a *single* proton of the  $\alpha\text{-CH}_3$  or  $\alpha\text{-CH}_2\text{OD}$  group of the substrate. The values of  $k_{\text{obsd}}$  for the deuterium exchange reactions of hydroxyacetone were not corrected for the presence of ca. 1% hydroxyacetone hydrate, because the correction is much smaller than the uncertainty in the rate constants for deuterium exchange which we estimate to be better than  $\pm 10\%$ .

$$\ln R = -k_{\text{obsd}}t \quad (3)$$

## Results

Figure 1 shows representative partial  $^1\text{H}$  NMR spectra of

### Scheme 3



hydroxyacetone obtained during deuterium exchange in the presence of acetate buffer and  $\text{Zn}^{2+}$  at pD 5.70 in  $\text{D}_2\text{O}$  at 25  $^\circ\text{C}$  and  $I = 1.0$  (KCl). The exchange for deuterium of the first  $\alpha\text{-CH}_3$  proton results in a decrease in the integrated area of the singlet at 2.058 ppm and the appearance of an upfield-shifted triplet ( $J_{\text{HD}} = 2.5$  Hz) at 2.040 ppm due to the  $\alpha\text{-CH}_2\text{D}$  group of the product. The exchange for deuterium of the first  $\alpha\text{-CH}_2\text{OD}$  proton results in a decrease in the integrated area of the singlet at 4.282 ppm and the appearance of upfield-shifted triplet ( $J_{\text{HD}} = 2.5$  Hz) at 4.259 ppm due to the  $\alpha\text{-CHDOD}$  group of the product.

First-order rate constants  $k_{\text{obsd}}$  ( $\text{s}^{-1}$ ) for exchange of a *single* proton of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone were determined from the slopes of semilogarithmic plots of reaction progress  $R$  (calculated from eqs 1 and 2, respectively) against time according to eq 3. The exchange for deuterium of *any* of the three  $\alpha\text{-CH}_3$  protons at hydroxyacetone converts the substrate with an  $\alpha\text{-CH}_3$  group to the product with an  $\alpha\text{-CH}_2\text{D}$  group (Scheme 3). Therefore, the first-order rate constant  $k_{\text{ex}}$  ( $\text{s}^{-1}$ ) for reaction of the  $\alpha\text{-CH}_3$  group is 3-fold larger than the rate constant for exchange of a *single*  $\alpha\text{-CH}_3$  proton, so that  $k_{\text{ex}}$

**Table 1.** First-Order Rate Constants  $k_{\text{ex}}$  (s<sup>-1</sup>) for the Deuterium Exchange Reaction of a Single  $\alpha$ -CH<sub>3</sub> Group of Acetone in the Presence of 0.36 M Acetate Buffer and Various Concentrations of Added Zinc Dication<sup>a</sup>

[Zn <sup>2+</sup> ] <sub>tot</sub> /M <sup>b</sup>	[AcO <sup>-</sup> ]/M <sup>c</sup>	10 <sup>8</sup> $k_{\text{ex}}$ /s <sup>-1</sup>
0.000	0.294	5.5
0.040	0.273	5.5
0.090	0.248	5.9
0.140	0.224	5.6
0.180	0.207	6.1

<sup>a</sup> For reactions at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). <sup>b</sup> Total concentration of zinc used to prepare the solution,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ . <sup>c</sup> The concentration of free acetate anion calculated from the total concentrations of acetate buffer and Zn<sup>2+</sup> used to prepare the solution at pD 5.70 and the values of  $\text{p}K_{\text{a}} = 5.06$  for acetic acid and  $K_{\text{assoc}} = 7.0 \text{ M}^{-1}$  for formation of the  $(\text{AcO}\cdot\text{Zn})^+$  complex determined in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl).

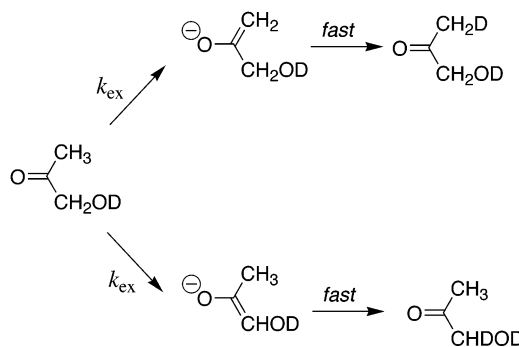
$= 3k_{\text{obsd}}$  (Scheme 3). Similarly,  $k_{\text{ex}} = 2k_{\text{obsd}}$  for reaction of the  $\alpha$ -CH<sub>2</sub>OD group of hydroxyacetone to give the  $\alpha$ -CHDOD group at the product (Scheme 3). The values of  $k_{\text{ex}}$  (s<sup>-1</sup>) for the deuterium exchange reactions of a *single*  $\alpha$ -CH<sub>3</sub> group of acetone were calculated from the values of  $k_{\text{obsd}}$  using a similar procedure, which was described in our earlier work.<sup>25</sup>

Table 1 gives values of  $k_{\text{ex}}$  (s<sup>-1</sup>) for the deuterium exchange reaction of a *single*  $\alpha$ -CH<sub>3</sub> group of acetone determined in the presence of 0.36 M acetate buffer and various concentrations of added Zn<sup>2+</sup> at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). Table 2 gives values of  $k_{\text{ex}}$  (s<sup>-1</sup>) for the deuterium exchange reactions of the  $\alpha$ -CH<sub>3</sub> and  $\alpha$ -CH<sub>2</sub>OD groups of hydroxyacetone determined in the presence of various concentrations of acetate buffer and added Zn<sup>2+</sup> at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). The concentrations of AcO<sup>-</sup>, AcOD, free Zn<sup>2+</sup>, and the  $(\text{AcO}\cdot\text{Zn})^+$  complex present in solution (Table 2) were calculated from the total concentrations of acetate buffer and Zn<sup>2+</sup> used to prepare the solution at pD 5.70 and the values of  $\text{p}K_{\text{a}} = 5.06$  for acetic acid and  $K_{\text{assoc}} = 7.0 \text{ M}^{-1}$  for formation

**Table 2.** First-order Rate Constants  $k_{\text{ex}}$  (s<sup>-1</sup>) for the Deuterium Exchange Reactions of the  $\alpha$ -CH<sub>2</sub>OD and  $\alpha$ -CH<sub>3</sub> Groups of Hydroxyacetone in the Presence of Various Concentrations of Acetate Buffer and Added Zinc Dication<sup>a</sup>

[Zn <sup>2+</sup> ] <sub>tot</sub> , M <sup>b</sup>	[Buffer] <sub>tot</sub> , M <sup>c</sup>	[AcO <sup>-</sup> ], M <sup>d</sup>	[AcOD], M <sup>d</sup>	[Zn <sup>2+</sup> ] <sub>free</sub> , M <sup>d</sup>	[(AcO·Zn) <sup>+</sup> ], M <sup>d</sup>	10 <sup>7</sup> $k_{\text{ex}}$ /s <sup>-1</sup> , $\alpha$ -CH <sub>2</sub> OD <sup>e</sup>	10 <sup>7</sup> $k_{\text{ex}}$ /s <sup>-1</sup> , $\alpha$ -CH <sub>2</sub> OD <sup>f</sup>	10 <sup>7</sup> $k_{\text{ex}}$ /s <sup>-1</sup> , $\alpha$ -CH <sub>3</sub> <sup>g</sup>	10 <sup>7</sup> $k_{\text{ex}}$ /s <sup>-1</sup> , $\alpha$ -CH <sub>3</sub> <sup>h</sup>
0.180	0.365	0.211	0.047	0.073	0.107	9.54	8.98	4.65	3.13
	0.250	0.133	0.030	0.094	0.087	6.90	6.37	3.39	2.16
	0.150	0.072	0.016	0.122	0.061	4.34	3.84	2.37	1.37
	0.045	0.019	0.004	0.161	0.021	1.48	1.00	1.26	0.460
0.140	0.365	0.228	0.051	0.054	0.086	7.18	6.71	3.84	2.42
	0.250	0.146	0.033	0.069	0.071	5.40	4.97	2.82	1.70
	0.150	0.081	0.018	0.091	0.051	3.24	2.84	1.83	0.957
	0.045	0.022	0.005	0.123	0.018	1.02	0.645	0.830	0.177
0.090	0.365	0.251	0.056	0.033	0.057	4.60	4.25	2.76	1.45
	0.250	0.164	0.037	0.042	0.048	3.58	3.27	2.04	1.06
	0.150	0.093	0.021	0.055	0.036	1.98	1.70	1.35	0.631
	0.045	0.026	0.006	0.077	0.014	0.600	0.354	0.540	0.0707
0.040	0.365	0.277	0.062	0.014	0.026	2.08	1.85	1.89	0.683
	0.250	0.186	0.042	0.017	0.023	1.44	1.25	1.28	0.412
	0.150	0.108	0.024	0.023	0.018	0.850	0.697	0.850	0.273
	0.045	0.031	0.007	0.033	0.007	0.252	0.133	0.312	0.0226
0.000	0.365	0.298	0.067	0.000	0.000	0.120		1.12	
	0.250	0.204	0.046	0.000	0.000	0.112		0.786	
	0.150	0.123	0.028	0.000	0.000	0.0480		0.480	
	0.045	0.037	0.008	0.000	0.000	0.0140		0.146	

<sup>a</sup> For reactions at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). <sup>b</sup> Total concentration of zinc used to prepare the solution,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ . <sup>c</sup> Total concentration of acetate buffer used to prepare the solution,  $[\text{Buffer}]_{\text{tot}} = \{[\text{AcOD}] + [\text{AcO}^-] + [(\text{AcO}\cdot\text{Zn})^+]\}$ . <sup>d</sup> The concentrations of AcO<sup>-</sup>, AcOD, free Zn<sup>2+</sup>, and the  $(\text{AcO}\cdot\text{Zn})^+$  complex present in solution were calculated from the total concentrations of acetate buffer and Zn<sup>2+</sup> used to prepare the solution at pD 5.70 and the values of  $\text{p}K_{\text{a}} = 5.06$  for acetic acid and  $K_{\text{assoc}} = 7.0 \text{ M}^{-1}$  for formation of the  $(\text{AcO}\cdot\text{Zn})^+$  complex determined in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). <sup>e</sup> Observed rate constant for deprotonation of the  $\alpha$ -CH<sub>2</sub>OD group of hydroxyacetone. <sup>f</sup> Observed rate constant for proton-transfer catalyzed by the combined action of zinc dication and acetate ion in a third-order reaction, calculated as  $k_{\text{ex}}' = (k_{\text{ex}} - k_0)$ , see text. <sup>g</sup> Observed rate constant for deprotonation of the  $\alpha$ -CH<sub>3</sub> group of hydroxyacetone.

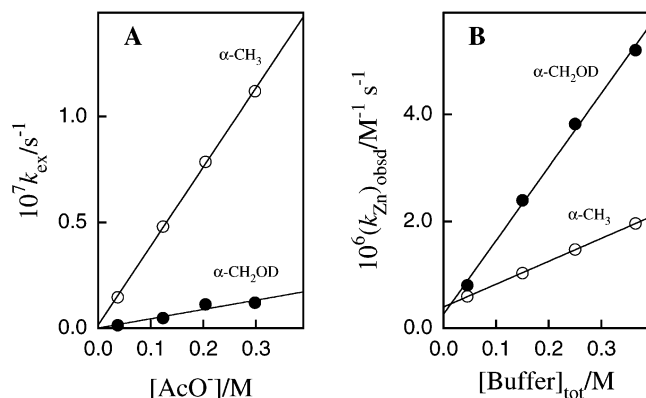
**Scheme 4**

of the  $(\text{AcO}\cdot\text{Zn})^+$  complex (Scheme 2) determined in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl) (see Experimental Section).

## Discussion

Deprotonation of the  $\alpha$ -carbonyl methyl and methylene groups of hydroxyacetone and acetone to form the corresponding enolates is the rate-limiting step for formation of the mono-deuterated functional groups in D<sub>2</sub>O (Scheme 4).<sup>14,25–27</sup> Therefore the rate constants  $k_{\text{ex}}$  (s<sup>-1</sup>) for deuterium exchange are the rate constants for deprotonation of the  $\alpha$ -CH<sub>3</sub> and  $\alpha$ -CH<sub>2</sub>OD groups of these substrates.

The evaluation of the rate equation for deprotonation of hydroxyacetone in aqueous solution in the presence of both acetate anion and zinc dication is complicated because there exist many different pathways for proton transfer. The most difficult problem is that zinc dication and acetate anion exist as a mixture of the free ions and the  $(\text{AcO}\cdot\text{Zn})^+$  complex which forms with an association constant of  $K_{\text{assoc}} = 7.0 \text{ M}^{-1}$  in D<sub>2</sub>O under our experimental conditions (Scheme 2). The acetate anion present in this complex is expected to be much less reactive as a Brønsted base toward deprotonation of carbon than is free



**Figure 2.** (A) Dependence of  $k_{\text{ex}}$  (s<sup>-1</sup>) for deprotonation of hydroxyacetone on the concentration of acetate anion in the absence of Zn<sup>2+</sup> at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). Key: (●) deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  group; (○) deprotonation of the  $\alpha\text{-CH}_3$  group. (B) Dependence of the observed second-order rate constants  $(k_{\text{Zn}})_{\text{obsd}}$  (M<sup>-1</sup> s<sup>-1</sup>) for deprotonation of hydroxyacetone catalyzed by Zn<sup>2+</sup> (determined as the slopes of the correlations in Figure 3) on the total concentration of acetate buffer at pD 5.70 in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). The y-intercepts of these correlations give the second-order rate constants  $k_{\text{Zn}}$  (M<sup>-1</sup> s<sup>-1</sup>) for deprotonation catalyzed by zinc dication. Key: (●) deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  group; (○) deprotonation of the  $\alpha\text{-CH}_3$  group.

acetate anion, as a result of the large effect of chelation on basicity.<sup>34</sup> However, it is not clear whether free Zn<sup>2+</sup> and the (AcO·Zn)<sup>+</sup> complex have similar electrophilic reactivities or if the presence of acetate anion in the coordination sphere of Zn<sup>2+</sup> reduces the electrophilicity of the metal ion. We have therefore compared the qualities of the fits of the data for deprotonation of hydroxyacetone (Table 2) to eq 4, which includes the single third-order term  $k_{\text{T}}$  for catalysis by acetate anion and total added Zn<sup>2+</sup>, and to eq 5, which includes explicit third-order terms  $k_{\text{T}}'$  and  $k_{\text{T}}''$  for reactions involving acetate anion and free Zn<sup>2+</sup> or the (AcO·Zn)<sup>+</sup> complex, respectively.

$$k_{\text{ex}} = k_0 + k_{\text{AcO}}[\text{AcO}^-] + k_{\text{Zn}}[\text{Zn}^{2+}]_{\text{tot}} + k_{\text{T}}[\text{AcO}^-][\text{Zn}^{2+}]_{\text{tot}} \quad (4)$$

$$k_{\text{ex}} = k_0 + k_{\text{AcO}}[\text{AcO}^-] + k_{\text{Zn}}[\text{Zn}^{2+}]_{\text{tot}} + k_{\text{T}}'[\text{AcO}^-][\text{Zn}^{2+}]_{\text{free}} + k_{\text{T}}''[\text{AcO}^-][(\text{AcO}^- \cdot \text{Zn})^+] \quad (5)$$

**Deprotonation of Hydroxyacetone. (1) Buffer-Catalyzed and Solvent-Catalyzed Reactions.** Figure 2A shows the dependence of  $k_{\text{ex}}$  (s<sup>-1</sup>) for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone on the concentration of

acetate anion in the absence of Zn<sup>2+</sup> at pD 5.70 in D<sub>2</sub>O (Table 2). The observed rate increase is due mainly to catalysis by acetate anion rather than acetic acid because the anion is the major form of the buffer at pD 5.70 ( $\text{p}K_{\text{a}} = 5.06$ ) and the values of  $k_{\text{HA}}$  (M<sup>-1</sup> s<sup>-1</sup>) for Brønsted general acid catalysis of deprotonation of  $\alpha\text{-carbonyl}$  carbon are typically 2–10-fold smaller than  $k_{\text{A}}$  (M<sup>-1</sup> s<sup>-1</sup>) for Brønsted general base catalysis.<sup>22</sup> The slopes of the correlations in Figure 2A give the second-order rate constants  $k_{\text{AcO}}$  (M<sup>-1</sup> s<sup>-1</sup>) for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone by acetate anion that are reported in Table 3.

The y-intercept of the correlation in Figure 2A for deprotonation of the  $\alpha\text{-CH}_3$  group of hydroxyacetone is  $k_0$  (s<sup>-1</sup>) for the uncatalyzed (solvent) reaction at pD 5.70 (Table 3). The contribution of this pathway for deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  group of hydroxyacetone is not detectable. These data are in qualitative agreement with the results of an earlier study which failed to detect deprotonation of methoxyacetone by water.<sup>23</sup>

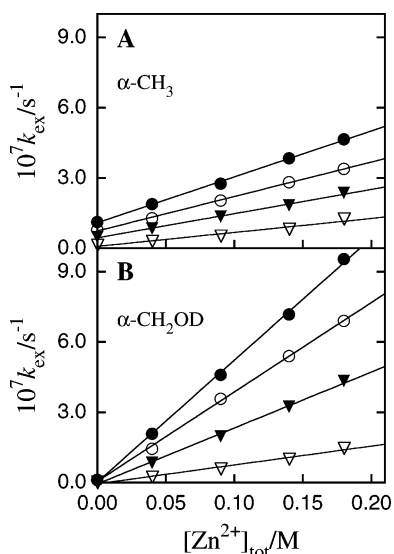
**(2) Bimolecular Catalysis by Zinc Dication.** Figure 3 shows linear plots of  $k_{\text{ex}}$  (s<sup>-1</sup>) for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone against the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}}$ , for experiments in which the total concentration of acetate buffer,  $[\text{Buffer}]_{\text{tot}} = \{[\text{AcOD}] + [\text{AcO}^-] + [(\text{AcO} \cdot \text{Zn})^+]\}$ , is held constant. The slopes of these correlations are equal to the observed second-order rate constants for zinc catalysis,  $(k_{\text{Zn}})_{\text{obsd}}$  (M<sup>-1</sup> s<sup>-1</sup>). The values of  $(k_{\text{Zn}})_{\text{obsd}}$  exhibit a strong dependence on the concentration of buffer and it is not possible to work at buffer concentrations where  $k_{\text{Zn}}$  (M<sup>-1</sup> s<sup>-1</sup>) is the dominant term in the rate law of eq 4. However, Figure 2B shows that the plots of  $(k_{\text{Zn}})_{\text{obsd}}$  (M<sup>-1</sup> s<sup>-1</sup>) against  $[\text{Buffer}]_{\text{tot}}$  are linear. The y-intercepts of the correlations in Figure 2B give the second-order rate constants  $k_{\text{Zn}}$  (M<sup>-1</sup> s<sup>-1</sup>) for Zn<sup>2+</sup>-assisted deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone in the absence of buffer that are reported in Table 3.

**(3) Third-Order Zinc-Dication-Catalyzed Reactions.** The dominant term in the rate law of eq 4 for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone is the third-order term  $k_{\text{T}}$  (M<sup>-2</sup> s<sup>-1</sup>) for the Zn<sup>2+</sup>-assisted proton transfer to acetate anion, which accounts for 7–67% and 53–94%, respectively, of the values of  $k_{\text{ex}}$  (s<sup>-1</sup>) reported in Table 2. This term was evaluated by subtracting the sum of the contributions of the solvent, AcO<sup>-</sup>-catalyzed and Zn<sup>2+</sup>-catalyzed reactions,

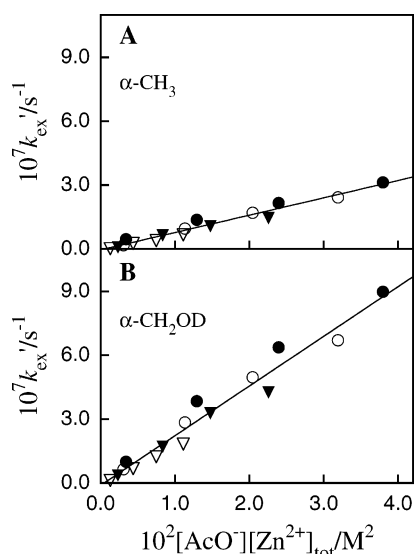
**Table 3.** Rate Constants for Deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  Groups of Hydroxyacetone Catalyzed by Acetate Anion and Zinc Dication<sup>a</sup>

catalyst	rate constant	$\alpha\text{-CH}_3$	$\alpha\text{-CH}_2\text{OD}$	$k_{\text{CH}_3}/k_{\text{CH}_2\text{OD}}^b$
solvent	$k_0/\text{s}^{-1c}$	$1.5 \times 10^{-9}$		
AcO <sup>-</sup>	$k_{\text{AcO}}/\text{M}^{-1} \text{s}^{-1d}$	$(3.7 \pm 0.1) \times 10^{-7}$	$(4.4 \pm 0.9) \times 10^{-8}$	$8.4 \pm 1.7$
Zn <sup>2+</sup>	$k_{\text{Zn}}/\text{M}^{-1} \text{s}^{-1e}$	$(4.0 \pm 0.1) \times 10^{-7}$	$(2.6 \pm 1.2) \times 10^{-7}$	
AcO <sup>-</sup> /total Zn <sup>2+</sup>	$k_{\text{T}}/\text{M}^{-2} \text{s}^{-1f}$	$(8.2 \pm 0.4) \times 10^{-6}$	$(2.3 \pm 0.1) \times 10^{-5}$	$0.36 \pm 0.02$
AcO <sup>-</sup> /free Zn <sup>2+</sup>	$k_{\text{T}}'/\text{M}^{-2} \text{s}^{-1g,h}$	$(1.2 \pm 0.1) \times 10^{-5}$	$(3.4 \pm 0.3) \times 10^{-5}$	$0.35 \pm 0.04$
AcO <sup>-</sup> /(AcO·Zn) <sup>+</sup>	$k_{\text{T}}''/\text{M}^{-2} \text{s}^{-1h,i}$	$(5.0 \pm 1.0) \times 10^{-6}$	$(1.4 \pm 0.3) \times 10^{-5}$	$0.36 \pm 0.11$

<sup>a</sup> For reactions in D<sub>2</sub>O at 25 °C and  $I = 1.0$  (KCl). The quoted errors are standard deviations. <sup>b</sup> Ratio of rate constants for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone. <sup>c</sup> First-order rate constant for solvent-catalyzed deprotonation at pD 5.70, determined from the y-intercept of the correlation in Figure 2A. <sup>d</sup> Second-order rate constant for deprotonation by acetate anion, determined as the slopes of the correlations in Figure 2A. <sup>e</sup> Second-order rate constant for deprotonation catalyzed by all forms of zinc in solution, determined as the y-intercepts of the correlations in Figure 2B. <sup>f</sup> Third-order rate constant for acetate-anion-promoted deprotonation catalyzed by all forms of zinc in solution, determined as the slopes of the correlations in Figure 4. This treatment assumes equal electrophilic reactivities of free Zn<sup>2+</sup> and the (AcO·Zn)<sup>+</sup> complex (see text). <sup>g</sup> Third-order rate constant for acetate-anion-promoted deprotonation catalyzed by free zinc dication. <sup>h</sup> Determined from the nonlinear least squares two-parameter fit of the data in Table 2 to eq 5 using the values of  $k_0$ ,  $k_{\text{AcO}}$ , and  $k_{\text{Zn}}$  given in this table. <sup>i</sup> Third-order rate constant for acetate-anion-promoted deprotonation catalyzed by the (AcO·Zn)<sup>+</sup> complex.



**Figure 3.** Dependence of  $k_{\text{ex}}$  ( $\text{s}^{-1}$ ) for deprotonation of hydroxyacetone on the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ , for experiments in which the total concentration of acetate buffer  $[\text{Buffer}]_{\text{tot}} = \{[\text{AcOD}] + [\text{AcO}^-] + [(\text{AcO}\cdot\text{Zn})^+]\}$  is held constant, at pD 5.70 in  $\text{D}_2\text{O}$  at 25 °C and  $I = 1.0$  (KCl). The slopes of these correlations give the observed second-order rate constants ( $k_{\text{Zn}}\text{obsd}$ ) ( $\text{M}^{-1} \text{s}^{-1}$ ) for deprotonation of hydroxyacetone catalyzed by  $\text{Zn}^{2+}$ . (A) Deprotonation of the  $\alpha\text{-CH}_3$  group. Key: (●)  $[\text{Buffer}]_{\text{tot}} = 0.365 \text{ M}$ ; (○)  $[\text{Buffer}]_{\text{tot}} = 0.250 \text{ M}$ ; (▼)  $[\text{Buffer}]_{\text{tot}} = 0.150 \text{ M}$ ; (▽)  $[\text{Buffer}]_{\text{tot}} = 0.045 \text{ M}$ . (B) Deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  group. Key: (●)  $[\text{Buffer}]_{\text{tot}} = 0.365 \text{ M}$ ; (○)  $[\text{Buffer}]_{\text{tot}} = 0.250 \text{ M}$ ; (▼)  $[\text{Buffer}]_{\text{tot}} = 0.150 \text{ M}$ ; (▽)  $[\text{Buffer}]_{\text{tot}} = 0.045 \text{ M}$ .



**Figure 4.** Dependence of  $k'_{\text{ex}} = (k_{\text{ex}} - k_o')$  ( $\text{s}^{-1}$ ) for deprotonation of hydroxyacetone on  $[\text{AcO}^-][\text{Zn}^{2+}]_{\text{tot}}$  at pD 5.70 in  $\text{D}_2\text{O}$  at 25 °C and  $I = 1.0$  (KCl), where  $[\text{Zn}^{2+}]_{\text{tot}}$  is the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ . The slopes of these correlations give the third-order rate constants  $k_{\text{T}}$  ( $\text{M}^{-2} \text{s}^{-1}$ ) for proton-transfer catalyzed by both acetate anion and all forms of zinc in solution. (A) Deprotonation of the  $\alpha\text{-CH}_3$  group. Key: (●)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.180 \text{ M}$ ; (○)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.140 \text{ M}$ ; (▼)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.090 \text{ M}$ ; (▽)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.040 \text{ M}$ . (B) Deprotonation of the  $\alpha\text{-CH}_2\text{OD}$  group. Key: (●)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.180 \text{ M}$ ; (○)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.140 \text{ M}$ ; (▼)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.090 \text{ M}$ ; (▽)  $[\text{Zn}^{2+}]_{\text{tot}} = 0.040 \text{ M}$ .

denoted by  $k_o'$  ( $\text{s}^{-1}$ , eq 6), from  $k_{\text{ex}}$  to give the values of  $k'_{\text{ex}} = (k_{\text{ex}} - k_o')$  ( $\text{s}^{-1}$ , eq 7) reported in Table 2. Figure 4 shows the linear plots of  $k'_{\text{ex}}$  against  $[\text{AcO}^-][\text{Zn}^{2+}]_{\text{tot}}$  for deprotonation of the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  groups of hydroxyacetone, where  $[\text{Zn}^{2+}]_{\text{tot}}$  is the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ . The slopes of these plots give the

third-order rate constants  $k_{\text{T}}$  ( $\text{M}^{-2} \text{s}^{-1}$ ) for proton transfer catalyzed by both acetate anion and all forms of zinc dication in solution that are reported in Table 3.

$$k_o' = k_o + k_{\text{AcO}}[\text{AcO}^-] + k_{\text{Zn}}[\text{Zn}^{2+}]_{\text{tot}} \quad (6)$$

$$k'_{\text{ex}} = (k_{\text{ex}} - k_o') = k_{\text{T}}[\text{AcO}^-][\text{Zn}^{2+}]_{\text{tot}} \quad (7)$$

The correlations in Figure 4 show that there is a fair fit of the experimental data to the rate law of eq 4, but that there are systematic positive deviations of  $k'_{\text{ex}}$  for reactions conducted at the highest total zinc concentration  $[\text{Zn}^{2+}]_{\text{tot}} = 0.180 \text{ M}$  (●), and systematic negative deviations for reactions conducted at the lowest total zinc concentration  $[\text{Zn}^{2+}]_{\text{tot}} = 0.040 \text{ M}$  (▽). These deviations provide evidence that the catalytic reactivity of free  $\text{Zn}^{2+}$  is higher than that of the  $(\text{AcO}\cdot\text{Zn})^+$  complex, because the ratio of the concentrations of free  $\text{Zn}^{2+}$  and the  $(\text{AcO}\cdot\text{Zn})^+$  complex increases with increasing  $[\text{Zn}^{2+}]_{\text{tot}}$  (Table 2).

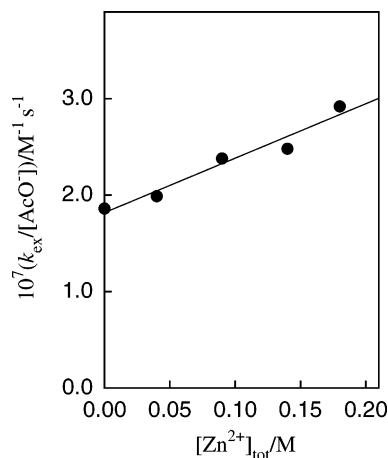
Table 3 reports third-order rate constants  $k_{\text{T}}'$  ( $\text{M}^{-2} \text{s}^{-1}$ ) and  $k_{\text{T}}''$  ( $\text{M}^{-2} \text{s}^{-1}$ ) for catalysis by free  $\text{Zn}^{2+}$  and the  $(\text{AcO}\cdot\text{Zn})^+$  complex, respectively, of the acetate-ion-promoted deprotonation of hydroxyacetone that were determined from the nonlinear least-squares two-parameter fits of the experimental data to eq 5, using the values of  $k_o$ ,  $k_{\text{AcO}}$ , and  $k_{\text{Zn}}$  given in Table 3. The values of  $k_{\text{T}}$  ( $\text{M}^{-2} \text{s}^{-1}$ ) determined by assuming equal catalytic reactivities of free  $\text{Zn}^{2+}$  and the  $(\text{AcO}\cdot\text{Zn})^+$  complex (eqs 4 and 7) lie between the values of  $k_{\text{T}}'$  ( $\text{M}^{-2} \text{s}^{-1}$ ) and  $k_{\text{T}}''$  ( $\text{M}^{-2} \text{s}^{-1}$ ) obtained by treating these species as separate catalytic entities. The latter model gives a 2-fold greater electrophilic reactivity of free  $\text{Zn}^{2+}$  than of the  $(\text{AcO}\cdot\text{Zn})^+$  complex, which is chemically plausible. Overall, these data support the conclusion that there is a  $\leq 2$ -fold difference in the catalytic activities of free  $\text{Zn}^{2+}$  and the  $(\text{AcO}\cdot\text{Zn})^+$  complex.

The three-parameter fits of the experimental data to eq 5 using the values of  $k_o$  and  $k_{\text{AcO}}$  given in Table 3 and treating  $k_{\text{Zn}}$ ,  $k_{\text{T}}'$ , and  $k_{\text{T}}''$  as independent parameters gave values of  $k_{\text{T}}'$  and  $k_{\text{T}}''$  ( $\text{M}^{-2} \text{s}^{-1}$ ) that are very similar to those obtained from the two-parameter fits described above.

**Deprotonation of Acetone.** Figure 5 shows the dependence of  $k_{\text{ex}}/[\text{AcO}^-]$  ( $\text{M}^{-1} \text{s}^{-1}$ ) on the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}}$ , in the presence of 0.36 M acetate buffer, where  $k_{\text{ex}}$  is the first-order rate constant for deprotonation of a single  $\alpha\text{-CH}_3$  group of acetone. Related data for deprotonation of hydroxyacetone (Figures 2A and 2B) show that at this high concentration of acetate buffer the contributions of  $k_o$  and  $k_{\text{Zn}}$  to the rate law (eq 4) are not significant so that  $k_{\text{ex}} = k_{\text{AcO}}[\text{AcO}^-] + k_{\text{T}}[\text{AcO}^-][\text{Zn}^{2+}]_{\text{tot}}$ . The slope of the plot in Figure 5 gives  $k_{\text{T}} = (5.7 \pm 0.7) \times 10^{-7} \text{ M}^{-2} \text{s}^{-1}$  as the third-order rate constant for deprotonation of a single  $\alpha\text{-CH}_3$  group of acetone by acetone catalyzed by all forms of zinc in solution that is reported in Table 4. Table 4 also reports statistically corrected rate constants for deprotonation of a single  $\alpha\text{-CH}_3$  group of acetone catalyzed by acetate anion and by the combined action of acetate anion and acetic acid that were calculated from literature data.<sup>21</sup>

**$\alpha\text{-OR}$  Substituent Effects on Proton Transfer from Carbon.** Hine and co-workers have reported that deprotonation of the  $\alpha\text{-CH}_3$  group of methoxyacetone by amine and phenoxide

(34) For example, the value of  $\text{p}K_{\text{a}} = 8.96$  for water bound to  $\text{Zn}^{2+}$  [Barnum, D. W. *Inorg. Chem.* **1983**, *22*, 2297–2305] is nearly 7 units lower than  $\text{p}K_{\text{a}} = 15.7$  for water.



**Figure 5.** Dependence of  $k_{\text{ex}}/[\text{AcO}^-]$  ( $\text{M}^{-1} \text{s}^{-1}$ ) for deprotonation of a single  $\alpha\text{-CH}_3$  group of acetone on the total concentration of added zinc,  $[\text{Zn}^{2+}]_{\text{tot}} = \{[\text{Zn}^{2+}]_{\text{free}} + [(\text{AcO}\cdot\text{Zn})^+]\}$ , in the presence of 0.36 M acetate buffer at pD 5.70 in  $\text{D}_2\text{O}$  at  $25^\circ\text{C}$  and  $I = 1.0$  (KCl).

bases is up to 6-fold faster than deprotonation of the  $\alpha\text{-CH}_2\text{-OMe}$  group, but that the  $\alpha\text{-CH}_2\text{OMe}$  group is slightly more reactive toward deprotonation by hydroxide ion.<sup>23</sup> By comparison, we observe an 8-fold larger value of  $k_{\text{AcO}}$  ( $\text{M}^{-1} \text{s}^{-1}$ ) for deprotonation of the  $\alpha\text{-CH}_3$  group than of the  $\alpha\text{-CH}_2\text{OD}$  group of hydroxyacetone by acetate anion (Table 4). Overall, these data show that the net effect of polar electron-withdrawing  $\alpha$ -substituents is to *destabilize* the carbanion-like transition state for deprotonation of  $\alpha$ -carbonyl carbon by general bases.

The value of  $k_{\text{AcO}}$  ( $\text{M}^{-1} \text{s}^{-1}$ ) for deprotonation of the  $\alpha\text{-CH}_3$  group of hydroxyacetone by acetate anion is 3.4-fold larger than that for deprotonation of a single  $\alpha\text{-CH}_3$  of acetone (Table 4). This is consistent with the expected polar effect of the  $-\text{OH}$  group when this electron-withdrawing substituent is placed distant from the reaction center. There should be a larger polar effect of the addition of an  $\alpha\text{-OH}$  group to acetone on  $k_{\text{AcO}}$  ( $\text{M}^{-1} \text{s}^{-1}$ ) for deprotonation of the  $\alpha\text{-CH}_2\text{OH}$  group of hydroxyacetone, where the substituent is attached directly to the acidic carbon. Assuming an attenuation factor of 3-fold per carbon atom,<sup>35</sup> the *predicted* polar effect of the addition of an  $\alpha\text{-OH}$  substituent to acetone is a ca.  $(3.4)(3^2)/1.5 = 20$ -fold increase in  $k_{\text{AcO}}$ .<sup>36</sup> In fact, the addition of an  $\alpha\text{-OH}$  group to acetone results in a 2.5-fold *decrease* in  $k_{\text{AcO}}$  for proton transfer to acetate anion (Table 4), so that deprotonation of the  $\alpha\text{-CH}_2\text{-OH}$  group of hydroxyacetone by acetate anion is ca. 50-fold slower than expected. This corresponds to a 2.3 kcal/mol *destabilization* of the transition state for proton transfer from carbon by an  $\alpha\text{-OH}$  group.

The destabilization of the transition state for deprotonation of  $\alpha$ -carbonyl carbon by  $\alpha\text{-OR}$  substituents reported here and by Hine and co-workers<sup>23,37</sup> might be a consequence either of a substituent effect on the thermodynamic driving force for proton transfer that is partly expressed in the transition state, or of a substituent effect on the Marcus intrinsic barrier for proton transfer.<sup>38–40</sup> Electron-withdrawing  $\alpha\text{-OR}$  substituents will stabilize negative charge that develops at the enolate ion,

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(36) This calculation includes a 1.5-fold statistical correction for the presence of three protons at an  $\alpha\text{-CH}_3$  group of acetone but only two protons at the  $\alpha\text{-CH}_2\text{OH}$  group of hydroxyacetone.

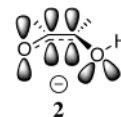
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**Table 4.** Rate Constants for Electrophilic Catalysis of the Deprotonation of a Single  $\alpha\text{-CH}_3$  Group of Acetone and the  $\alpha\text{-CH}_3$  and  $\alpha\text{-CH}_2\text{OD}$  Groups of Hydroxyacetone by Acetate Anion<sup>a</sup>

Substrate	Base	Electrophile	Rate Constant	$k_{\text{rel}}^b$	$\Delta\Delta G^\ddagger$ kcal/mol <sup>c</sup>
	AcO <sup>-</sup>	D <sub>2</sub> O	$1.1 \times 10^{-7}$ $\text{M}^{-1} \text{s}^{-1 \text{d}}$		
	AcO <sup>-</sup>	AcOD	$5.3 \times 10^{-8}$ $\text{M}^{-2} \text{s}^{-1 \text{e}}$	0.48	1.9
	AcO <sup>-</sup>	Zn <sup>2+</sup>	$5.7 \times 10^{-7}$ $\text{M}^{-2} \text{s}^{-1 \text{f}}$	5.2	3.3
	AcO <sup>-</sup>	D <sub>2</sub> O	$3.7 \times 10^{-7}$ $\text{M}^{-1} \text{s}^{-1 \text{f}}$		
	AcO <sup>-</sup>	Zn <sup>2+</sup>	$1.2 \times 10^{-5}$ $\text{M}^{-2} \text{s}^{-1 \text{f}}$	32	4.4
	AcO <sup>-</sup>	D <sub>2</sub> O	$4.4 \times 10^{-8}$ $\text{M}^{-1} \text{s}^{-1 \text{f}}$		
	AcO <sup>-</sup>	Zn <sup>2+</sup>	$3.4 \times 10^{-5 \text{f}}$ $\text{M}^{-2} \text{s}^{-1}$	770	6.3

<sup>a</sup> Determined in this work for reactions in  $\text{D}_2\text{O}$  at  $25^\circ\text{C}$  and  $I = 1.0$  (KCl), unless noted otherwise. <sup>b</sup> The increase in rate upon the addition of 1.0 M of the electrophilic catalyst (AcOD or  $\text{Zn}^{2+}$ ), calculated as the ratio of the respective third-order and second-order rate constants. <sup>c</sup> The stabilization of the transition state for the termolecular reaction by the electrophilic catalyst at 298 K, calculated using a common standard state of 1 M water and 1 M electrophilic catalyst. <sup>d</sup> Calculated from  $k_{\text{AcO}} = 2.1 \times 10^{-7} \text{M}^{-1} \text{s}^{-1}$  for deprotonation of acetone by acetate anion in water at  $25^\circ\text{C}$  and  $I = 2.0$  ( $\text{KNO}_3$ ) [ref 21], a solvent isotope effect of unity [ref 21], and a 2-fold statistical correction for the presence of two  $\alpha\text{-CH}_3$  groups at acetone. <sup>e</sup> Calculated from  $k_{\text{T}} = 2.1 \times 10^{-7} \text{M}^{-2} \text{s}^{-1}$  for deprotonation of acetone by acetate anion catalyzed by acetic acid in water at  $25^\circ\text{C}$  and  $I = 2.0$  ( $\text{KNO}_3$ ) [ref 21], a solvent isotope effect of 2.0 [ref 21], and a statistical correction for the presence of two  $\alpha\text{-CH}_3$  groups at acetone. <sup>f</sup> This work.

they will destabilize the  $\text{C}=\text{C}$  double bond at the enolate product relative to the  $\text{C}-\text{C}$  single bond at the reactant,<sup>41</sup> and there will be a direct *repulsive* interaction between the electron lone pairs at the  $\alpha\text{-OH}$  group and the delocalized electron pair at the enolate (see structure 2).<sup>42</sup> The observation that destabilizing



interactions dominate the observed  $\alpha\text{-OR}$  substituent effect is consistent with the notion that there is a strong repulsive interaction between the electron lone pairs at the  $\alpha\text{-OH}$  group

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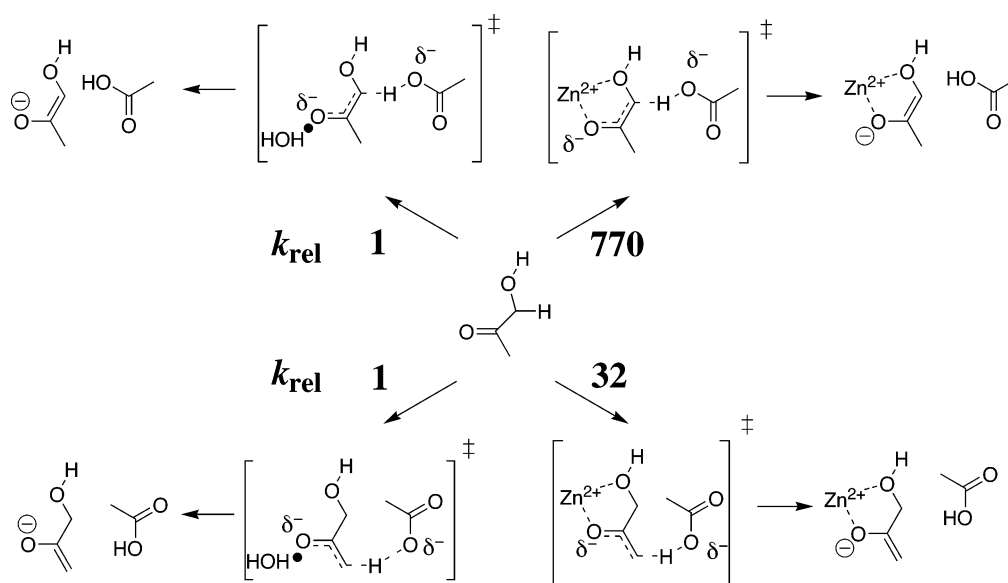
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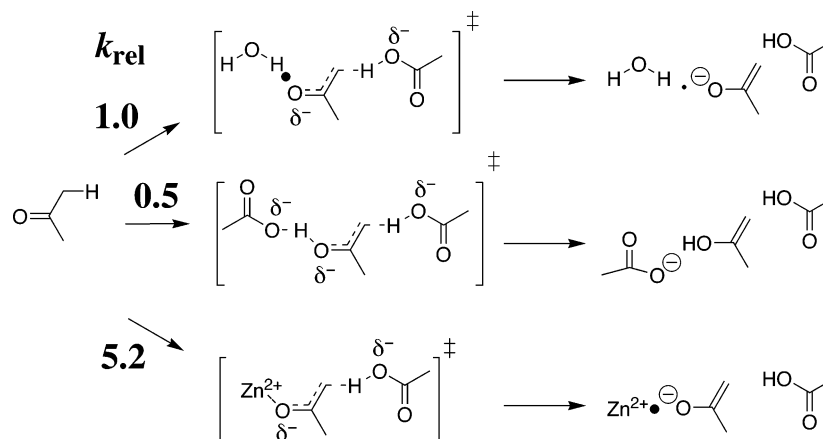
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(42) Hine, J. *Structural Effects on Equilibria in Organic Chemistry*; John Wiley & Sons: New York, 1975; pp 181–184.

Scheme 5



Scheme 6



and the delocalized electron pair at the enolate (see 2). The transition state for proton transfer from  $\alpha$ -carbonyl carbon to acetate anion is more crowded than the enolate product and may be further destabilized by interactions between the electron-rich base catalyst and an  $\alpha$ -OR substituent. Such a destabilizing interaction would be specific for the transition state and would therefore be reflected in an increase in the Marcus intrinsic barrier for proton transfer.

The balance of these opposing interactions on the equilibrium constant for deprotonation of  $\alpha$ -carbonyl carbon in water is not known. However, the results of a recent computational study of proton transfer in the gas phase show that the addition of a single  $\alpha$ -F substituent to acetaldehyde results in a 7.0 kcal/mol decrease in  $\Delta G_0$  for deprotonation to form the enolate, while the addition of second  $\alpha$ -F substituent results in a 4.3 kcal/mol increase in  $\Delta G_0$ .<sup>43</sup> Therefore, the destabilizing interactions between the negative charge at the enolate and an  $\alpha$ -F substituent dominate as the  $\alpha$ -carbon becomes more highly substituted.

**Stabilization of the Transition State for Deprotonation of  $\alpha$ -Carbonyl Carbon by Electrophilic Reagents.** The third-order rate constants  $k_T$  for the Zn<sup>2+</sup>-assisted acetate-ion-

promoted deprotonation of the  $\alpha$ -CH<sub>3</sub> and  $\alpha$ -CH<sub>2</sub>OH groups of hydroxyacetone are 32-fold and 770-fold larger, respectively, than the corresponding second-order rate constants  $k_{\text{AcO}}$  for proton transfer to acetate anion “assisted” by solvent water that is present at 55 M (Scheme 5 and Table 4). This shows that Zn<sup>2+</sup> stabilizes the transition state for proton transfer from the  $\alpha$ -CH<sub>3</sub> and  $\alpha$ -CH<sub>2</sub>OH groups of hydroxyacetone by 4.4 and 6.3 kcal/mol, respectively, relative to a common standard state of 1 M water and 1 M Zn<sup>2+</sup> (Table 4).

There are only small differences between the second-order rate constant for deprotonation of acetone by acetate anion and the third-order rate constants for catalysis of this reaction by acetic acid and Zn<sup>2+</sup>, so that the stabilities of the transition states for the acetate-ion-promoted reactions assisted by Zn<sup>2+</sup>, acetic acid, and solvent water (55 M) are similar (Scheme 6 and Table 4). Acetic acid and Zn<sup>2+</sup> stabilize the transition state for proton transfer from acetone to acetate anion by 1.9 and 3.3 kcal/mol, respectively, relative to a common standard state of 1 M water and 1 M electrophile (Table 4).

The larger stabilization of the transition state for deprotonation of hydroxyacetone (4.4 or 6.3 kcal/mol) than of that for deprotonation of acetone (3.3 kcal/mol) by interaction with zinc dication is due to the additional “chelate interaction” between

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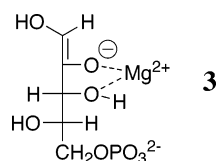
the metal cation and the  $\alpha$ -OH group at the substrate. Previous studies of metal ion catalysis of deprotonation of  $\alpha$ -carbonyl carbon have focused on the reactions of relatively strong carbon acids containing pyridine and related nitrogen heterocycles as substituents that can serve as a chelation site for the metal ion catalyst.<sup>17–19</sup> The results reported here show that for the simplest ketone acetone the absence of a chelation site renders metal ion catalysis of proton transfer only marginally more effective than electrophilic catalysis by small Brønsted acids such as acetic acid.

We attribute the greater assistance by 1 M  $\text{Zn}^{2+}$  of the acetate-ion-promoted deprotonation of the  $\alpha$ - $\text{CH}_2\text{OH}$  group (770-fold) than of the  $\alpha$ - $\text{CH}_3$  group (32-fold) of hydroxyacetone (Scheme 5) to the following related effects:

(1) A greater stabilization of the transition state for deprotonation of the  $\alpha$ - $\text{CH}_2\text{OH}$  group than of the  $\alpha$ - $\text{CH}_3$  group from direct electrostatic interactions between  $\text{Zn}^{2+}$  and the acetate anion base which presumably lies closer to the metal cation in the transition state for the former reaction. Similar differences in electrostatic interactions between the developing enolate anion and Brønsted base catalysts of different charge type have been proposed to explain the ca. 10-fold greater reactivity of neutral amines than of oxyanion bases of the same  $\text{p}K_a$  as catalysts of deprotonation of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate.<sup>44</sup>

(2) The relief of destabilizing interactions between the electron lone pairs at the  $\alpha$ -OH group of hydroxyacetone and the acetate anion base associated with the formation of a chelate between the  $\alpha$ -OH group and  $\text{Zn}^{2+}$ . This should result in an increase in  $k_T$  for deprotonation of the  $\alpha$ - $\text{CH}_2\text{OH}$  group of hydroxyacetone relative to  $k_{\text{AcO}}$ , but it should not affect the relative values of these rate constants for deprotonation of the  $\alpha$ - $\text{CH}_3$  group.

**Enzymatic Catalysis of Proton Transfer at Carbon.** Part of the activation of hydroxyacetone toward  $\text{Zn}^{2+}$ -catalyzed proton transfer is a result of the stabilizing chelate interaction between the hydroxy group at the substrate and the metal dication that is expressed at transition state for proton transfer. X-ray crystallographic analysis of complexes between 3-keto-L-gulonate 6-phosphate decarboxylase and analogues of the 1,2-enediolate reaction intermediate provide evidence that the essential magnesium dication is coordinated to the C-2 oxygen and the nonreacting C-3 hydroxyl of the reaction intermediate (see 3).<sup>45</sup> This interaction is also expected to be expressed at



the Michaelis complex and at the transition state for the enzyme-catalyzed decarboxylation reaction. It would be surprising if this simple interaction were not utilized to stabilize the transition state for other metalloenzyme-catalyzed reactions of sugars.

The similar stabilization of the transition state for the acetate-ion-promoted deprotonation of acetone by acetic acid (1.9 kcal/mol, Table 4) and by  $\text{Zn}^{2+}$  (3.3 kcal/mol, Table 4) shows that

there is only a modest advantage to the use of a metal dication rather than a Brønsted acid to stabilize the transition state for deprotonation of the  $\alpha$ -carbonyl carbon of acetone. This might have been predicted. Similar enzyme-catalyzed proton-transfer reactions proceed with assistance by either Brønsted acids<sup>10,11</sup> or metal ions.<sup>9,20</sup> This shows that these two types of electrophiles function equally well in enzymatic catalysis of the deprotonation of  $\alpha$ -carbonyl carbon, and it provides evidence that there is no large energetic advantage that favors the evolution of protein catalysts of proton transfer that use a particular type of electrophile.

The rate accelerations associated with electrophilic catalysis of proton transfer from acetone to acetate anion by 1 M acetic acid or 1 M  $\text{Zn}^{2+}$  in solution (Scheme 6) are very small in comparison to those observed for enzyme catalysis, so that electrophilic catalysis of proton transfer at an enzyme active site must be “enhanced” if it is to make a significant contribution to an enzymatic rate acceleration. For example: (1) there is good evidence that the transition state for triosephosphate isomerase-catalyzed enolization of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate is stabilized by interaction between the substrate carbonyl group and a neutral His-95.<sup>10</sup> (2) The results of computational studies are consistent with the conclusion that the rate acceleration for enolase-catalyzed deprotonation of the weakly acidic carbon of 2-phosphoglycerate arises mainly from the greater electrostatic stabilization of the putative dianionic enolate intermediate by interactions with two magnesium dications, compared with the corresponding interactions between the metal dications and the carboxylate monoanion at bound substrate.<sup>46–48</sup>

Enhancement of electrophilic catalysis will result when the stabilizing interactions between the transition state and the catalytic group(s) are strengthened as these species are moved from aqueous solution to an enzyme active site. In the case of electrophilic catalysis of deprotonation of  $\alpha$ -carbonyl carbon by acetic acid in water, transition state stabilization results from the partial expression of the ca. 8 kcal/mol driving force for proton transfer from acetic acid ( $\text{p}K_a = 4.8$ ) to the enolate of acetone ( $\text{p}K_a = 10.9$  for the enol oxygen)<sup>49</sup> in the transition state.<sup>12,50</sup> For catalysis by  $\text{Zn}^{2+}$ , the stabilization is due to electrostatic interactions between the metal dication and the developing negative charge at the carbonyl/enolate oxygen in the transition state.<sup>6</sup> The change from the polar solvent water to an “organized” enzyme active site<sup>51,52</sup> with an “effective” dielectric constant substantially smaller than that of water provides a general mechanism for the enhancement of electrophilic catalysis of deprotonation of  $\alpha$ -carbonyl carbon by both types of electrophilic catalysts for the following reasons.

(1) The change from water to an enzyme active site with an “effective” dielectric constant smaller than that of water will result in an increase in the basicity of the enolate anion product. This will favor catalysis of deprotonation of  $\alpha$ -carbonyl carbon

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provided there is a net increase in the driving force for proton transfer from the Brønsted acid catalyst to the enolate oxyanion that is expressed in the transition state.<sup>12</sup> However, a change in the dielectric constant of the medium should not lead to large change in the *relative* pK<sub>a</sub>s of the carboxylic acid catalyst and the enolate, because both oxyanions will be destabilized in a solvent of low dielectric constant. Rather, a change in their relative pK<sub>a</sub> would require “tuning” of the interactions of the two basic sites with the protein catalyst. This could occur, for example, by differential hydrogen bonding or through a “gradient” in the local dielectric constant of the active site.<sup>51,52</sup>

(2) In the absence of a favorable thermodynamic driving force for proton transfer to the enolate the transition state for its formation will be stabilized mainly by formation of a hydrogen bond to an acid catalyst.<sup>3,4</sup> There is controversy about the structure of such hydrogen bonds in water and at enzymes.<sup>3,5,7,53–56</sup> Partial proton transfer from the hydrogen bond donor to the acceptor will have the effect of spreading negative charge over two basic sites, and this will be favored for any hydrogen bond at an enzyme active site of low dielectric constant where the formation of “localized” charge is unfavorable. The “delocalization” of charge will be greatest for a single-potential hydrogen bond, where the proton is bonded equally to the hydrogen bond donor and acceptor. Therefore, the formation of such hydrogen bonds will be favored at an enzyme active site of low dielectric constant.<sup>53–55</sup>

(3) In an active site of low dielectric constant an enolate oxyanion may be strongly stabilized “internally” by interactions with a covalently attached cationic group.<sup>57,58</sup> This has been proposed to occur for catalysis of the racemization of cationic

amino acids through zwitterionic enolate intermediates,<sup>28,59</sup> and for the related reaction catalyzed by pyruvate decarboxylase.<sup>60</sup>

(4) Electrophilic catalysis by metal cations is strongly attenuated in the polar solvent water and will be favored at an active site of low dielectric constant that enhances stabilizing electrostatic interactions between opposing charges.<sup>6</sup> This effect of changing the dielectric constant of the medium will be particularly large for interactions between dications and the transition state for the enolase-catalyzed deprotonation of 2-phosphoglycerate to give a dianionic enolate.<sup>46–48,61</sup>

Finally, it must be emphasized that the energetic cost of moving a polar transition state from solvent water to a nonpolar enzyme active site must be “paid for” through the development of tight binding interactions between the protein catalyst and the nonreacting parts of the substrate.<sup>62</sup>

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