Mechanistic Imperatives for Aldose−Ketose Isomerization in Water: Specific, General Base- and Metal Ion-Catalyzed Isomerization of Glyceraldehyde with Proton and Hydride Transfer

R. W. Nagorski[1] and John P. Richard*[,2]

Contribution from the Department of Chemistry, University at Buffalo, SUNY, Buffalo, New York 14260-3000

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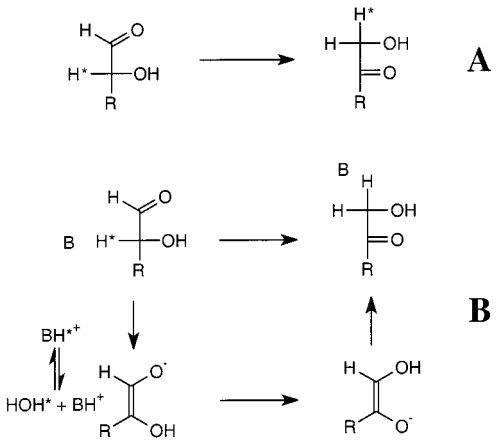
Abstract: The deuterium enrichment of dihydroxyacetone obtained from the aldose−ketose isomerization of D,L-glyceraldehyde in D_2O at 25 °C was determined by ^1H NMR spectroscopy from the integrated areas of the signals for the α-CH_2 and α-CHD groups of the product. One mole equivalent of deuterium is incorporated into the product when the isomerization is carried out in 150 mM pyrophosphate buffer at pD 8.4, but only 0.6 mol equiv of deuterium is incorporated into the product of isomerization in the presence of 0.01 M deuterioxide ion, so that 40% of the latter isomerization reaction proceeds by the intramolecular transfer of hydride ion. Several pathways were identified for catalysis of the isomerization of glyceraldehyde to give dihydroxyacetone. The isomerization with hydride transfer is strongly catalyzed by added Zn^{2+}. Deprotonation of glyceraldehyde is rate-determining for isomerization with proton transfer, and this proton-transfer reaction is catalyzed by Brønsted bases. Proton transfer also occurs by a termolecular pathway with catalysis by the combined action of Brønsted bases and Zn^{2+}. These results show that there is no large advantage to the spontaneous isomerization of glyceraldehyde in alkaline solution with either proton or hydride transfer, and that effective catalytic pathways exist to stabilize the transition states for both of these reactions in water. The existence of separate enzymes that catalyze the isomerization of sugars with *hydride* transfer and the isomerization of sugar phosphates with *proton* transfer is proposed to be a consequence of the lack of any large advantage to reaction by either of these pathways for the corresponding nonenzymatic isomerization in water.

Aldose−ketose isomerization is a simple but mechanistically ambiguous reaction which involves the formal transfer of H_2 from C-2 and O-2 to C-1 and O-1 of an α-hydroxy aldehyde to form the corresponding α-hydroxy ketone (Scheme 1). One hydrogen is transferred as a proton between the electronegative oxygens of the hydroxyl and carbonyl groups of the aldose. By contrast, transfer of the second hydrogen from C-2 to C-1 may occur directly as a hydride ion (Scheme 1A) or as a proton, in which case the bonding electron pair at C-2 moves through the carbon skeleton to C-1 (Scheme 1B). The question of the preferred pathway for this reaction in water has important implications in both chemistry and biology.

(1) The many studies on base-catalyzed deprotonation of α-carbonyl carbon in water to form enolates create a strong prejudice that these isomerization reactions occur by a proton-transfer mechanism in this solvent (Scheme 1B),[3] and this mechanism has been well documented for the general-base-catalyzed isomerization of glyceraldehyde 3-phosphate to give dihydroxyacetone phosphate in water.[4] However, the situation is more complicated. There is good evidence that the closely related Meerwein−Ponndorf−Verley reaction proceeds with intramolecular transfer of hydride in organic solvents,[5] and there are reports that this reaction mechanism (Scheme 1A) is viable

Scheme 1



for aldose−ketose isomerization in alkaline aqueous solution.[6] Furthermore, there is precedent for metal ion catalysis of intramolecular and intermolecular transfer of hydride ion from

(1) Present Address: Department of Chemistry, Illinois State University, Normal, IL 61790-4160.

(2) Telephone: (716) 645-6800, ext. 2194. Fax: (716) 645-6963. E-mail: jrichard@chem.buffalo.edu.

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(6) The small amount of intramolecular transfer of tritium observed during *anaerobic* hydroxide-ion-catalyzed epimerization of [2-^{3}H]-D-ribose to arabinose may occur by consecutive isomerization reactions with hydride transfer [Gleason, W. B.; Barker, R. *Can. J. Chem.* **1971**, *49*, 1433−1440]. More recent experimental evidence supports a proton-transfer mechanism for the hydroxide-ion-catalyzed isomerization of aldoses to ketoses. No intramolecular transfer of tritium was detected during isomerization of [2-^{3}H]-D-glucose to D-fructose [Isbell, H. S. *Adv. Chem. Ser.* **1973**, *117*, 70−87], and there is evidence that the interconversion of D-glucose, D-mannose, and D-fructose in alkaline D_2O proceeds through a common enolate reaction intermediate [Kooyman, C.; Vellenga, K.; de Wilt, H. G. J. *Carbohydr. Res.* **1977**, *54*, 33−44].

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α-hydroxy carbon to the carbonyl group in organic solvents,[7−9] but no work to determine whether a similar pathway is involved in the zinc-catalyzed isomerization of mannose 6-phosphate to give fructose 6-phosphate in water.[10] This lack of clarity in our understanding of the mechanism for these reactions is addressed in the present work aimed at determining the relative barriers to aldose−ketose isomerization in water with proton and hydride transfer.

(2) A guiding tenet for the interpretation of experimental studies of small-molecule models for enzymatic catalysis of *polar* reactions is that the enzymatic reaction will follow one of the mechanisms observed for the nonenzymatic process in water. A corollary to this is that separate enzymes with different reaction mechanisms will evolve only in cases where the barriers for the corresponding uncatalyzed reactions in water are similar, so that there is no large advantage to reaction by either pathway. The observation that enzymatic catalysis of isomerization of sugar phosphates proceeds by a proton-transfer mechanism,[11,12] while catalysis of isomerization of sugars proceeds with hydride transfer,[13−16] presents an interesting test for this corollary. The existence of enzymes that catalyze aldose−ketose isomerization by distinct proton and hydride transfer mechanisms suggests that these two reactions proceed with similar ease in water.

We have reported in preliminary form the results of studies on the aldose−ketose isomerization reaction of D,L-glyceraldehyde in dilute aqueous alkali.[17] We report here the full details of this earlier work, together with the results of additional experiments which show that there are several pathways for specific base, general base and metal ion catalysis of isomerization of glyceraldehyde with proton and hydride transfer and that the observed reaction in water depends on the relative concentrations of these catalytic reagents.

Experimental Section

Materials. Deuterium oxide (99.9% D) and deuterium chloride (35% w/w, 99.5% D) were purchased from Cambridge Isotope Laboratories, and KOD (40 wt %, 98+% D) was purchased from Aldrich. D,L-Glyceraldehyde (∼97%) and dihydroxyacetone dimer were purchased from Sigma and were used without further purification. Potassium pyrophosphate, potassium acetate, hexafluoroacetone hydrate, and pivalic acid were purchased from Aldrich and were used without further purification. Zinc chloride (99%) was purchased from Baker and was used without further purification.

General Methods. Solution pD was determined at 25 °C using an Orion model 720A pH meter equipped with a Radiometer GK2321C combination electrode. Values of pD were obtained by adding 0.40 to the observed reading of the pH meter.[18]

For reactions in the presence of KOD the ionic strength was maintained at 0.10 using KCl. Except for pyrophosphate, the ionic strength for reactions in buffered solution was maintained at 1.0 using KCl. Buffered solutions were prepared by dissolving the buffer component, KCl, and $ZnCl_2$ in D_2O followed by the addition of a measured amount of DCl or KOD to give the desired pD. The ratio of the concentrations of the acid and base forms of the buffer in these solutions was calculated from the solution pD and the following apparent pK_a's in D_2O at 25 °C and $I = 1.0$ (KCl) that were determined from the observed pD of solutions containing known concentrations of the buffer acid and its conjugate base: CH_3CO_2D, 5.1; $(CH_3)_3CCO_2D$, 5.4; $(CF_3)_2C(OD)_2$, 6.9.

A complex forms between Zn^{2+} and acetate ion that removes the free anion from acetate buffers and results in a decrease in the buffer ratio [AcO^-]/[AcOD] and a decrease in solution pD upon the addition of Zn^{2+}. A value of $K_{as} = 7\ M^{-1}$ for formation of the $AcO^-{\cdot}Zn^{2+}$ complex in D_2O 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^-] calculated from the decrease in the pD of acetate buffers upon addition of Zn^{2+}.[19] This is similar to $K_{as} = 7.9\ M^{-1}$ under the same conditions in H_2O.[20]

Solutions of glyceraldehyde and dihydroxyacetone (0.03−0.16 M, $I = 0.1$ or 1.0, KCl) were prepared and allowed to stand at room temperature for at least 1 day before use, or until only the monomeric substrates were detected by ^{1}H NMR spectroscopy. ^{1}H NMR analysis showed that 0.15% dihydroxyacetone was present as a contaminant in our commercial sample of D,L-glyceraldehyde.

^{1}H NMR Analyses. ^{1}H NMR spectra were generally recorded at 500 MHz in D_2O at 25 °C on a Varian Unity Inova 500 spectrometer. In some experiments to monitor deuterium incorporation into dihydroxyacetone ^{1}H NMR spectra were recorded at 400 MHz in D_2O at 25 °C on a Varian Unity Inova 400 spectrometer. Relaxation times for protons of the free carbonyl and hydrated forms of glyceraldehyde and dihydroxyacetone were determined as $T_1 = 4-6$ s using 10 mM solutions of substrate at $I = 1.0$ (KCl). ^{1}H NMR spectra were recorded using a spectral width of 5300 Hz, a 90° pulse angle, a 6 s acquisition time, and a relaxation delay between pulses of ≥ 54 s. Chemical shifts are reported relative to HOD at 4.65 ppm. Baselines were subjected to a first-order drift correction before determination of integrated peak areas.

The extents of hydration of glyceraldehyde and dihydroxyacetone in D_2O at 25 °C ($I = 1.0$, KCl) were determined to be 95 and 16%, respectively, by comparison of the integrated areas of appropriate signals for the free carbonyl and hydrated forms of these substrates. The relative concentrations of glyceraldehyde, ^{1}H-dihydroxyacetone (**H-DHA**) and ^{2}H-dihydroxyacetone (**D-DHA**) in mixtures of these compounds were determined from the integrated areas of the signals for the C-1 proton of glyceraldehyde hydrate (doublet, A_{CH}), the protons of the α-CH_2 groups of the keto forms of **H-DHA** and **D-DHA** (singlet, A_{CH2}), and the proton of the α-CHD group of the keto form of **D-DHA** (triplet, A_{CHD}) using eqs 1, 2, and 3, respectively. These areas were normalized to give relative total concentrations of glyceraldehyde, **H-DHA**, and **D-DHA** by dividing the observed area of the peak for the C-1 proton of glyceraldehyde hydrate by the fraction of glyceraldehyde present as the hydrate form (0.95, eq 1), and by dividing the area of the peak for the α-CH_2 protons of the keto form of dihydroxyacetone by the fraction of **DHA** present in the keto form (0.84 = [1−0.16], eqs 2 and 3). The values of A_{CH2} in eq 2 were corrected for the presence of the small (0.15%) amount of **H-DHA** that was present as a contaminant in glyceraldehyde at zero time. The ratios of normalized peak areas were reproducible to ±5%.

$$A_{GA} = A_{CH}/0.95 \quad (1)$$

$$A_{H-DHA} = (A_{CH2} - 2A_{CHD})/(4*0.84) \quad (2)$$

$$A_{D-DHA} = A_{CHD}/0.84 \quad (3)$$

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Kinetic Analyses. All reactions were carried out in D_2O at 25 °C.

Isomerization of Glyceraldehyde. The following procedure was used to monitor the aldose−ketose isomerization reactions of glyceraldehyde in the presence of KOD. Reactions were initiated by dilution of a 30 mM solution of glyceraldehyde into 10 mL of alkaline D_2O to give a final substrate concentration of 1.0 mM ($I = 0.10$, KCl). After a timed interval the reaction was quenched by adjusting the pD to 4−5 with 1 M DCl, and the solution was concentrated to ∼1 mL under reduced pressure on a rotary evaporator at <30 °C. The sample was transferred to an NMR tube and stored at 4 °C. It was shown in control experiments that no isomerization of glyceraldehyde or incorporation of deuterium into dihydroxyacetone occurred during the quenching and concentration of these solutions, or upon storage for up to one month at 4 °C. ^{1}H NMR spectra of these samples were generally obtained within one week of their preparation.

A similar procedure was used for reactions in the presence of 150 mM pyrophosphate buffer at pD 8.4, except the final substrate concentration was 1.5 mM and the reactions were quenched by adjusting the pD to 2−3. It was shown in control experiments that no isomerization of glyceraldehyde or incorporation of deuterium into dihydroxyacetone occurred during the quenching and concentration of these solutions or upon storage for 10 h at 4 °C. ^{1}H NMR spectra of these samples were obtained within 10 h of their preparation.

The aldose−ketose isomerization reactions of glyceraldehyde in buffered solutions containing Zn^{2+} were initiated by dilution of 0.2 mL of a 0.15 M solution of glyceraldehyde to 2.0 mL to give a final substrate concentration of 15 mM. Half of this solution was transferred to an NMR tube and was used to monitor the isomerization of glyceraldehyde by ^{1}H NMR. The remaining sample was used to monitor the solution pD. These runs were discontinued if a change in pD of ≥0.05 units was observed.

The progress of the isomerization of glyceraldehyde was monitored by ^{1}H NMR, and the fraction of glyceraldehyde remaining was calculated from the normalized integrated areas of the signals due to the reactant and products (eqs 1−3) according to eq 4.

$$f_{GA} = \frac{A_{GA}}{A_{GA} + A_{H-DHA} + A_{D-DHA}} \quad (4)$$

The observed first-order rate constants $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde were determined as the slope of plots of ln f_{GA} against time. The reactions were generally monitored during the first 10% of isomerization, except for reactions in the presence of KOD which were followed during the first 5% of isomerization.

Deuterium Exchange into Dihydroxyacetone. The procedures used to follow the deuterium exchange reactions of dihydroxyacetone ([S] = 1 mM) in the presence of 0.01 M KOD ($I = 0.10$, KCl) or 150 mM pyrophosphate buffer at pD 8.4 were identical to those used to monitor isomerization of glyceraldehyde under the same reaction conditions. The progress of the deuterium exchange reaction was monitored by ^{1}H NMR. Values of R, which is a measure of the reaction progress, were calculated according to eq 5

$$R = \frac{A_{CH2}}{A_{CH2} + A_{CHD}} \quad (5)$$

$$\ln R = -k_{obsd}t \quad (6)$$

where A_{CH2} is the integrated area of the singlet due to the α-CH_2 groups of **H-DHA** and **D-DHA**, and A_{CHD} is the integrated area of the triplet due to the α-CHD group of **D-DHA**. The reactions were generally followed during exchange of 10−30% of the first proton of each α-CH_2 group of the substrate. Semilogarithmic plots of reaction progress, R, against time according to eq 6 were linear with negative slopes equal to k_{obsd}, where k_{obsd} (s^{-1}) is the rate constant for exchange of a *single* α-proton of dihydroxyacetone.[21,22] The values of k_{obsd} were reproducible to ±10%.

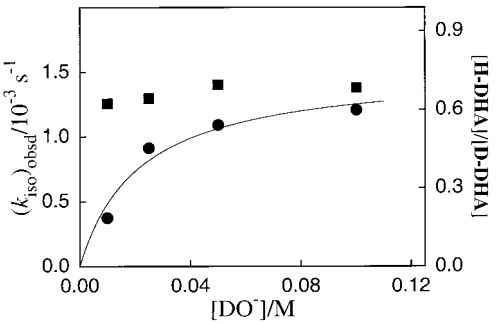

Figure 1. Dependence of the observed rate constant and the product distribution for isomerization of glyceraldehyde on the concentration of deuterioxide in D_2O at 25 °C and $I = 0.10$ (KCl). (●) Observed first-order rate constants $(k_{iso})_{obsd}$ for isomerization. The solid line shows the fit of the data to eq 8 derived for the mechanism shown in Scheme 3. (■) Ratio of the yields of the fully protonated product **H-DHA** and the monodeuterated product **D-DHA** (Scheme 2).

Results

^{1}H NMR analysis shows that besides dihydroxyacetone, the major products of reaction of 10 mM D-glyceraldehyde in D_2O buffered with 50 mM carbonate buffer at pD 10.2 are fructose and sorbose derived from aldol condensation.[23a] ^{1}H NMR analysis of the products of reaction of 10 mM glyceraldehyde in 0.01−0.10 M KOD in D_2O ($I = 0.1$, KCl) revealed complex signals that were therefore presumed to be due to the products of bimolecular aldol condensation.[23b] The product yields from aldol condensation decreased relative to those from unimolecular isomerization with decreasing concentration of the glyceraldehyde substrate. Dihydroxyacetone was the sole product detected by ^{1}H NMR during the first 10% of isomerization of 1.5 mM glyceraldehyde in D_2O buffered with 150 mM potassium pyrophosphate at pD 8.4 at 25 °C, and during the first 5% of isomerization of 1.0 mM glyceraldehyde in alkaline D_2O ([DO^-] = 0.01−0.10 M) at 25 °C ($I = 0.10$, KCl). The importance of aldol condensation is greatly decreased for reactions of glyceraldehyde in buffered solutions containing Zn^{2+} at pD 4.8−7.6, due to the strong catalysis of isomerization by Zn^{2+} (see below). Under these conditions, only isomerization products were detected by ^{1}H NMR during the first 10% of reaction of 15 mM glyceraldehyde at 25 °C ($I = 1.0$, KCl).

Figure 1 (●) shows the dependence of the observed rate constant $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde on the concentration of deuterioxide ion in D_2O at 25 °C ($I = 0.1$, KCl), determined by monitoring the formation of dihydroxyacetone by ^{1}H NMR. A value of $(k_{iso})_{obsd} = 1.6 \times 10^{-5}$ s^{-1} was determined for isomerization of glyceraldehyde in the presence of 150 mM potassium pyrophosphate in D_2O at pD 8.4 and 25 °C.

^{1}H NMR analyses of the products of isomerization of glyceraldehyde to give dihydroxyacetone in D_2O showed a singlet at ∼4.4 ppm due to the α-CH_2 groups of **H-DHA** and **D-DHA** with area A_{CH2}, and a triplet shifted 0.024 ppm upfield from this singlet due to the α-CHD group of **D-DHA** with area A_{CHD} (Scheme 2).[24] Figure 2 (●) shows that there is a decrease in A_{CH2}/A_{CHD} determined for the isomerization of glyceraldehyde in the presence of 0.01 M KOD at 25 °C ($I = 0.1$, KCl) with increasing extent of its conversion to dihydroxyacetone. The ratio $(A_{CH2}/A_{CHD})_o = 4.5 \pm 0.5$ for the *initial products* of the

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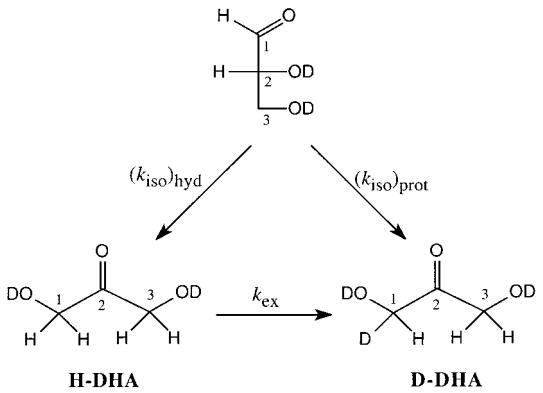
(24) Representative ^{1}H NMR spectra of mixtures of **H-DHA** and **D-DHA** were shown in Figure 1 of the preliminary report of this work [ref 17].

Scheme 2



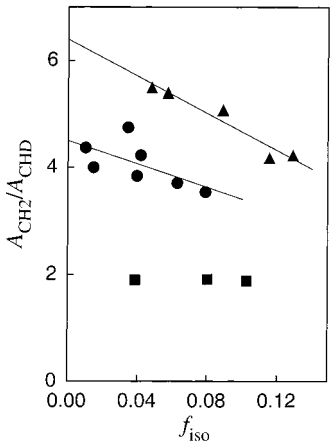


Figure 2. The change in the ratio of the integrated areas A_{CH2}/A_{CHD} of the ^{1}H NMR signals for the α-CH_2 and α-CHD groups of the dihydroxyacetone products with increasing extent of isomerization of glyceraldehyde in D_2O at 25 °C. (●) Isomerization in the presence of 0.01 M KOD (I = 0.10, KCl). (■) Isomerization in the presence of 150 mM potassium pyrophosphate buffer at pD 8.4. (▲) Isomerization in the presence of 0.15 M acetate buffer and 115 mM Zn^{2+} at pD 5.7 (I = 1.0, KCl).

isomerization reaction in 0.01 M KOD was determined by making a short linear extrapolation of the data to zero time. Figure 2 (■) shows that the change from 0.01 M KOD to 150 mM potassium pyrophosphate buffer at pD 8.4 results in a significant decrease to $A_{CH2}/A_{CHD} \approx 2$ that is essentially constant with time.[24]

The ratio of the yields of **H-DHA** and **D-DHA** (Scheme 2) were calculated from the limiting peak ratio $(A_{CH2}/A_{CHD})_o$ using eq 7 (cf. eqs 2 and 3). Values of [**H-DHA**]/[**D-DHA**] = 0.63 ± 0.1 and [**H-DHA**]/[**D-DHA**] ≈ 0 were determined for isomerization of glyceraldehyde in the presence of 0.01 M KOD and 150 mM potassium pyrophosphate buffer at pD 8.4, respectively. Figure 1 (■) shows that the values of [**H-DHA**]/[**D-DHA**] for isomerization of glyceraldehyde in alkaline D_2O, determined by extrapolation of product data to zero time, remain essentially constant in the presence of increasing [DO^-] in the range 0.01−0.10 M (I = 0.1, KCl), with an average value of [**H-DHA**]/[**D-DHA**] = 0.66 ± 0.1.

$$\frac{[\textbf{H-DHA}]}{[\textbf{D-DHA}]} = \frac{(A_{CH2})_o - (2A_{CHD})_o}{4(A_{CHD})_o} \qquad (7)$$

The decrease in A_{CH2}/A_{CHD} for isomerization of glyceraldehyde in the presence of 0.01 M KOD with increasing reaction time (Figure 2, ●) shows that there is washout of hydrogen from dihydroxyacetone due to base-catalyzed exchange with deuterium from solvent (k_{ex}, Scheme 2). Observed first-order rate constants for deuterium exchange into dihydroxyacetone were determined in control experiments in which exchange of the first proton of the α-CH_2 groups of dihydroxyacetone was monitored by ^{1}H NMR spectroscopy. Deuterium exchange results in decay of the singlet at ∼4.39 ppm due to the α-CH_2 groups of **H-DHA**, and appearance of an upfield triplet at ∼4.37 ppm due to the α-CHD group of **D-DHA**, in which the remaining α-proton is coupled to the α-deuterium ($J_{HD} \approx 2$ Hz).[25−27] Values of R, a measure of the progress of deuterium exchange, were calculated from the integrated areas of the signals due to the α-CH_2 and α-CHD groups of substrate and product according to eq 5, and rate constants k_{obsd} (s^{-1}) for exchange of a *single* α-proton of dihydroxyacetone were determined according to eq 6.[21,22] The reaction of **H-DHA** to give monodeuterated product **D-DHA** occurs at four times the rate of exchange of a single proton of **H-DHA**, so that $k_{ex} = 4k_{obsd}$, where k_{ex} (s^{-1}) is the first-order rate constant for exchange of the first α-proton of dihydroxyacetone.

Values of $k_{ex} = 1.7 \times 10^{-3}$ and 2.7×10^{-5} s^{-1} were determined for the deuterium exchange reaction of dihydroxyacetone in the presence of 0.01 M KOD (I = 0.1, KCl) and 150 mM potassium pyrophosphate buffer at pD 8.4 in D_2O at 25 °C, respectively. These rate constants are 4.5-fold and 1.7-fold larger than the rate constants $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde under the same reaction conditions. These data show that the rate constant for deuterium exchange into **H-DHA** in 0.01 M KOD is too small to account for the observed formation of **D-DHA** from glyceraldehyde during the early stages of isomerization (Scheme 2). For example, under these reaction conditions only 4% of **H-DHA** undergoes deuterium exchange to give **D-DHA** during the time required for isomerization of 1% of glyceraldehyde, but the yield of **D-DHA** during the first 1% of isomerization of glyceraldehyde is ∼60% ([**H-DHA**]/[**D-DHA**] = 0.63, see above).

Figure 3 shows the dependence of $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde on the concentration of added Zn^{2+} in weakly buffered solutions ([buffer]$_{tot}$ = 40 mM) in D_2O at 25 °C and I = 1.0 (KCl). The data are given in Table S1 of the Supporting Information. ^{1}H NMR analyses showed that **H-DHA** is the sole product of isomerization of glyceraldehyde under these conditions, so that isomerization in the presence of metal ions and low concentrations of buffer occurs essentially exclusively with intramolecular transfer of hydride. The slopes of the linear correlations of the data in Figure 3 were multiplied by $(1 + K_{hydrate})$, where $K_{hydrate} = 19$ is the equilibrium constant for hydration of the reactive carbonyl form of glyceraldehyde (see Experimental Section), to give apparent second-order rate constants $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) for Zn^{2+}-catalyzed isomerization of glyceraldehyde with intramolecular transfer of hydride. Figure 4 shows the pD-rate profile of the values of $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) that were determined from the data in Figure 3 and Table S1.

Table 1 gives the values of $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde in the presence of 0.15 and 0.31 M acetate buffer at pD 5.7 and 0−150 mM added Zn^{2+} in D_2O at 25 °C and I = 1.0 (KCl). ^{1}H NMR analysis showed that isomerization

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(27) Richard, J. P.; Nagorski, R. W. *J. Am. Chem. Soc.* **1999**, *121*, 4763−4770.

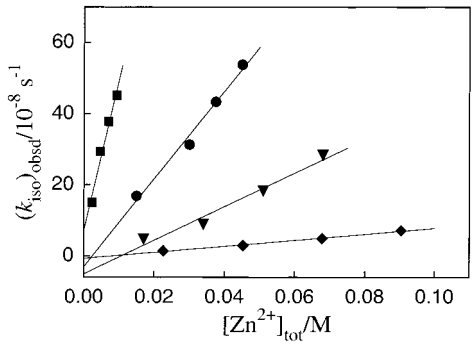


Figure 3. Dependence of the observed first-order rate constant $(k_{iso})_{obsd}$ for isomerization of glyceraldehyde with intramolecular hydride transfer on the total concentration of added Zn^{2+} at 25 °C and $I = 1.0$ (KCl). (■) Reactions in the presence of 40 mM hexafluoroacetone hydrate buffer at pD 7.6. (●) Reactions in the presence of 40 mM hexafluoroacetone hydrate buffer at pD 6.9. (▼) Reactions in the presence of 40 mM pivalate buffer at pD 6.4. (◆) Reactions in the presence of 40 mM acetate buffer at pD 5.7. The slopes of the linear correlations of the data were multiplied by $(1 + K_{hydrate}) = 20$ to give the apparent second-order rate constants $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) for Zn^{2+}-catalyzed isomerization of glyceraldehyde with hydride transfer.

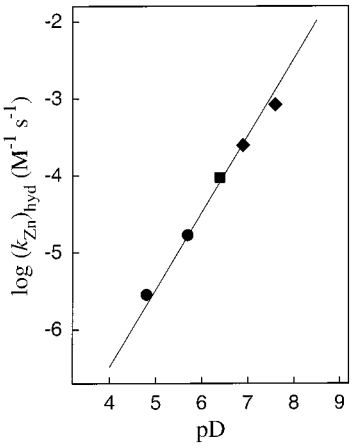


Figure 4. pD-rate profile of the apparent second-order rate constants $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) for Zn^{2+}-catalyzed isomerization of glyceraldehyde with intramolecular hydride transfer in D_2O at 25 °C and $I = 1.0$ (KCl). (●) Second-order rate constants determined for reactions in solutions buffered with 40 mM acetate. (■) Second-order rate constant determined for reaction in solution buffered with 40 mM pivalate. (◆) Second-order rate constants determined for reactions in solutions buffered with 40 mM hexafluoroacetone hydrate. The solid line has a slope of 1.0 and shows the fit to eq 9 which gives $(k_T)_{hyd} = 1.9 \times 10^4$ M^{-2} s^{-1} as the third-order rate constant for isomerization of glyceraldehyde with hydride transfer catalyzed by both DO^- and Zn^{2+} (Scheme 6A).

of glyceraldehyde in the absence of added Zn^{2+} at these relatively high buffer concentrations gives **D-DHA** as the sole product. However, in the presence of both acetate ion and Zn^{2+} mixtures of **H-DHA** and **D-DHA** are obtained as the products of isomerization. Figure 2 (▲) shows the decrease in A_{CH2}/A_{CHD} with increasing reaction time for the dihydroxyacetone product of isomerization of glyceraldehyde in the presence of 0.15 M acetate buffer at pD 5.7 and 115 mM added Zn^{2+}. A value of $(A_{CH2}/A_{CHD})_o = 6.4 \pm 0.5$ was determined from a linear extrapolation of the product data to zero time, which was used to calculate the product ratio [**H-DHA**]/[**D-DHA**] = 1.1 ± 0.1 (eq 7). Table 1 gives the values of [**H-DHA**]/[**D-DHA**] for isomerization of glyceraldehyde in the presence of 0.15 and 0.31 M acetate buffer at pD 5.7 and 0−150 mM added Zn^{2+} in D_2O at 25 °C and $I = 1.0$ (KCl) determined by this procedure.

Discussion

Reaction Pathways for Isomerization of Glyceraldehyde in Alkaline Solution. Figure 1 (●) shows that the observed first-order rate constants $(k_{iso})_{obsd}$ for isomerization of glyceraldehyde in alkaline D_2O at 25 °C ($I = 0.1$, KCl) increase with increasing [DO^-] to a limiting value of $\sim 10^{-3}$ s^{-1}. These data show that there is specific base catalysis of isomerization of glyceraldehyde by DO^-. We attribute the downward curvature at high pD to ionization of an O-1 hydroxyl of glyceraldehyde hydrate (the predominant species, $K_{hydrate} = 19$) to form the hydrate oxyanion ($(K_a)_{O1}$, Scheme 3), which, like the hydrate, is unreactive toward isomerization. A similar leveling off at high pH has been observed in the pH-rate profile for the elimination reaction of glyceraldehyde 3-phosphate, which proceeds by rate-limiting deprotonation of the substrate at C-2.[4] The solid line in Figure 1 shows the nonlinear least-squares fit of the data to eq 8, derived for the mechanism in Scheme 3, which gives $K_b = K_w/(K_a)_{O1} = 0.02$ M, and $k_{DO} = 1.4$ M^{-1} s^{-1} as the overall second-order rate constant for DO^--catalyzed isomerization of glyceraldehyde.[28a]

$$(k_{iso})_{obsd} = \frac{k_{DO}[DO^-]}{1 + K_{hydrate}(1 + \{(K_a)_{O1}[DO^-]/K_w\})} \quad (8)$$

Figure 1 (■) shows that the ratio of product yields [**H-DHA**]/[**D-DHA**] from isomerization of glyceraldehyde in alkaline D_2O at 25 °C and $I = 0.1$ (KCl) is independent of [DO^-] in the range 0.01−0.10 M. These data require that the first-order rate constants for isomerization by the competing proton and hydride transfer pathways have a similar dependence on [DO^-] and show similar decreases upon ionization of the hydrate at high pD (Scheme 3). Values of $(k_{DO})_{prot} = 0.8$ M^{-1} s^{-1} and $(k_{DO})_{hyd} = 0.6$ M^{-1} s^{-1} for DO^--catalyzed isomerization of glyceraldehyde with proton transfer and intramolecular transfer of hydride ion (Scheme 3) were calculated from the average product ratio [**H-DHA**]/[**D-DHA**] = 0.66 and the relationship $k_{DO} = (k_{DO})_{prot} + (k_{DO})_{hyd} = 1.4$ M^{-1} s^{-1}.

The formation of **D-DHA** occurs by deprotonation of glyceraldehyde by DO^- [$(k_{DO})_{prot}$, Scheme 4)], fast dilution of HOD in D_2O (k_{reorg}), and slower protonation of the enolate by D_2O (k_{DOD}). The following are consistent with the conclusion that the rate constants for isomerization of glyceraldehyde by the proton-transfer pathway to give **D-DHA** are essentially equal to the rate constants for deprotonation of this substrate at C-2 to give the enediolate.

(1) Formation of the enediolate from deprotonation of glyceraldehyde by DO^- is expected to be essentially irreversible, since the closely related intermediate generated by deprotonation of glyceraldehyde 3-phosphate partitions preferentially to dihydroxyacetone phosphate.[4] This reflects the marked thermodynamic driving force for this aldose−ketose isomerization reaction.[28b] This is consistent with our failure to detect

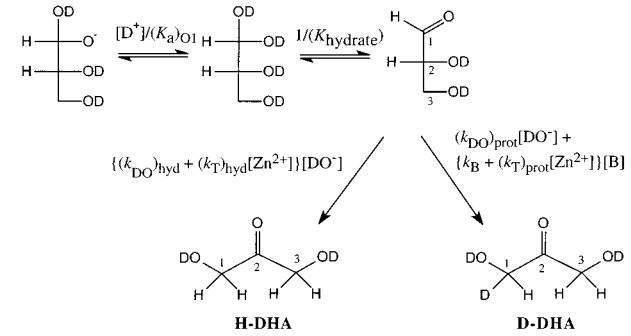
(28) (a) The value of $K_b = 0.02$ M can be combined with $K_w = 10^{-14.87}$ M^2 for the ionization constant of D_2O at 25 °C [Covington, A. K.; Robinson, R. A.; Bates, R. G. *J. Phys. Chem.* **1966**, *70*, 3820−3824] to give $(pK_a)_{O1} = 13.2$ for ionization of an O-1 hydroxyl of glyceraldehyde hydrate in D_2O. By comparison $pK_a = 13$ was determined for ionization of an O-1 hydroxyl of the hydrate of glyceraldehyde 3-phosphate in H_2O from the position of the downward break at high pH in the pH-rate profile for deprotonation to form the enediolate phosphate [ref 4]. (b) A value of $K_{eq} = 22$ has been determined as the overall equilibrium constant for interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [Veech, R. L.; Raijman, L.; Dalziel, K.; Krebs, H. A. *Biochem. J.* **1969**, *115*, 837−842]. Triosephosphate isomerase catalyzes the conversion of >90% of D-glyceraldehyde to dihydroxyacetone, so that $K_{eq} > 10$ for this reaction [Amyes, T. L., unpublished results].

Table 1. Rate Constants and Product Ratios for Isomerization of Glyceraldehyde To Give Dihydroxyacetone in the Presence of Acetate Buffers and Added Zn^{2+} at pD 5.7 in D_2O[a]

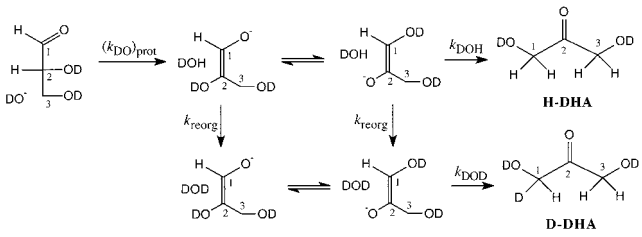
$[buffer]_{tot}$ (M)[b]	$[Zn^{2+}]_{tot}$ (M)[c]	$(k_{iso})_{obsd}/10^{-8}$ (s^{-1})[d]	**[H-DHA]/[D-DHA]**[e]	$(k_{iso})_{prot}/10^{-8}$ (s^{-1}) [f]	$(k_{iso})_{hyd}/10^{-8}$ (s^{-1}) [g]	$(k_{Zn})_{prot}$ $(M^{-1}\,s^{-1})$[h]	$(k_{Zn})_{hyd}$ $(M^{-1}\,s^{-1})$[i]
0.15	0.000	2.6	0	2.6	0		
	0.038	6.3	0.94	3.2	3.0		
	0.076	10.6	1.03	5.2	5.4	1.0×10^{-5}	1.4×10^{-5}
	0.115	14.1	1.10	6.7	7.4		
	0.153	21.8	1.03	10.8	11.1		
0.31	0.000	6.8	0	6.8	0		
	0.038	13.4	0.24	10.8	2.6		
	0.076	18.6	0.48	12.6	6.0	2.0×10^{-5}	1.6×10^{-5}
	0.114	26.3	0.48	17.8	8.5		
	0.152	35.1	0.56	22.5	12.6		

[a] At 25 °C and $I = 1.0$ (KCl). [b] Total concentration of acetate buffer at pD 5.7. [c] Total concentration of Zn^{2+} added as $ZnCl_2$. [d] Observed first-order rate constant for isomerization of glyceraldehyde determined by monitoring the appearance of dihydroxyacetone by ^1H NMR. [e] The ratio of the yields of **H-DHA** and **D-DHA** determined by ^1H NMR (Scheme 2). [f] Rate constant for isomerization of glyceraldehyde with proton transfer to give **D-DHA** calculated from $(k_{iso})_{obsd}$ and the fractional yield of **D-DHA** using eq 10. [g] Rate constant for isomerization of glyceraldehyde with hydride transfer to give **H-DHA** calculated from $(k_{iso})_{obsd}$ and the fractional yield of **H-DHA** using eq 11. [h] Apparent second-order rate constant for Zn^{2+}-catalyzed isomerization of glyceraldehyde with proton transfer determined from the slope of the plot of $(k_{iso})_{prot}$ against $[Zn^{2+}]$ and $K_{hydrate} = 19$ (see text). [i] Apparent second-order rate constant for Zn^{2+}-catalyzed isomerization of glyceraldehyde with hydride transfer determined from the slope of the plot of $(k_{iso})_{hyd}$ against $[Zn^{2+}]$ and $K_{hydrate} = 19$ (see text).

Scheme 3



Scheme 4



significant incorporation of deuterium into unreacted glyceraldehyde during the course of isomerization in alkaline D_2O or in the presence of 150 mM pyrophosphate buffer at pD 8.4.[29]

(2) The value of $(k_{DO})_{prot} = 0.8\ M^{-1}\ s^{-1}$ for DO^--catalyzed isomerization of glyceraldehyde to give **D-DHA** at 25 °C is similar to $(k_{HO})_{prot} = 5.6\ M^{-1}\ s^{-1}$ determined for deprotonation of glyceraldehyde 3-phosphate by HO^- at the higher temperature of 39 °C.[4]

(3) Isomerization of glyceraldehyde in D_2O in the presence of 150 mM potassium pyrophosphate buffer at pD 8.4 occurs essentially exclusively by a proton-transfer pathway to form **D-DHA** ($A_{CH2}/A_{CHD} \approx 2$, Figure 2). We attribute the increase in the rate of isomerization with proton transfer over that with hydride transfer under these conditions to classic Brønsted general base catalysis of deprotonation of C-2 of glyceraldehyde. This reaction has been extensively characterized in an earlier study of the reactions of glyceraldehyde 3-phosphate.[4]

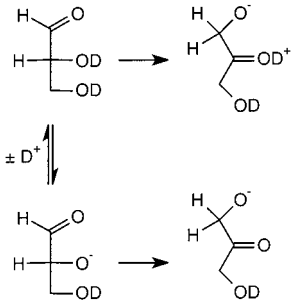
(29) We did not follow these isomerization reactions for extended times because of competing aldol condensation, and this limited our ability to detect any deuterium exchange that may occur into unreacted glyceraldehyde.

The formation of **H-DHA** must occur by direct intramolecular transfer of hydrogen as hydride ion. The alternative stepwise proton-transfer mechanism through a carbanion reaction intermediate has been observed for related base-catalyzed reactions in organic solvents such as *tert*-butyl alcohol, where the lifetime of ion pair or ion dipole intermediates is sufficiently long to allow for a *conducted tour* of a proton derived from substrate between basic sites at the reaction intermediate.[30a] However, the conducted tour reaction mechanism is less likely to occur for reactions in aqueous solvents, where ion pair and ion dipole intermediates of organic reactions undergo fast diffusional separation to free species.[30b] It is not a viable mechanism for isomerization of glyceraldehyde with intramolecular transfer of hydrogen (Scheme 4), because the enediolate intermediate of this reaction undergoes relatively slow protonation to form dihydroxyacetone ($k_{DOH} < 10^5\ s^{-1}$, Scheme 4).[30c] Therefore, the molecule of DOH that forms by deprotonation of glyceraldehyde by DO^- will undergo dilution with solvent (D_2O) much faster than protonation by DOH to form **H-DHA**. This dilution may occur by reorganization of DOH within the solvation shell that surrounds the reaction intermediate ($k_{reorg} \approx 10^{11}\ s^{-1}$, Scheme 4),[30d] or by somewhat slower diffusional separation to free species ($k_d \approx 10^{10}\ s^{-1}$).[30b]

Isomerization of glyceraldehyde with hydride transfer is formally specific-base-catalyzed because ionization of the C-2 hydroxyl group to give the C-2 oxyanion greatly increases the rate and driving force for intramolecular transfer of hydride ion to C-1.[5,7−9] This reflects the greater stability of the neutral C-2

(30) (a) Ford, W. T.; Cram, D. J. *J. Am. Chem. Soc.* **1968**, *90*, 2606−2611. Jaeger, D. A.; Broadhurst, M. D.; Cram, D. J. *J. Am. Chem. Soc.* **1979**, *101*, 717−732. (b) Richard, J. P.; Tsugi, Y. *J. Am. Chem. Soc.* **2000**, *122*, 3963−3964. Richard, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1984**, *106*, 1373−1383. (c) A value of $k_{HOH} = 5 \times 10^4\ s^{-1}$ has been determined for protonation of the enolate of acetone by the bulk solvent water [Chiang, Y.; Kresge, A. J.; Tang, Y. S.; Wirz, J. *J. Am. Chem. Soc.* **1984**, *106*, 460−462]. The rate constant for reaction of a single proton of a molecule of solvent will be smaller than this and will depend on the number of reactive water molecules that surround the enolate. Similar rate constants are expected for protonation of the enolates of acetone and dihydroxyacetone because similar rate constants have been determined for base-catalyzed deprotonation of the CH_3 and CH_2OMe groups of methoxyacetone [Hine, J.; Hampton, K. G.; Menon, B. C. *J. Am. Chem. Soc.* **1967**, *89*, 2664−2668] and of the CH_3 and CH_2OH groups of hydroxyacetone [Crugeiras, J., unpublished results]. (d) This corresponds to the rate constant for the dielectric relaxation of water [Giese, K.; Kaatze, U.; Pottel, R. *J. Phys. Chem.* **1970**, *74*, 3718−3725. Kaatze, U. *J. Chem. Eng. Data* **1989**, *34*, 371−374. Kaatze, U.; Pottel, R.; Schumacher, A. *J. Phys. Chem.* **1992**, *96*, 6017−6020].

Scheme 5

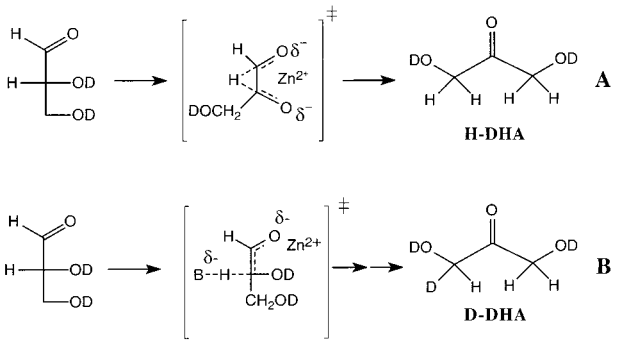


carbonyl group formed from reaction of the C-2 oxyanion than of the protonated ketone formed from reaction of the neutral alcohol (Scheme 5). We prefer a stepwise mechanism for isomerization with hydride transfer through the C-2 oxyanion intermediate over a concerted mechanism in which proton transfer from the C-2 hydroxyl to DO^- and intramolecular transfer of hydride ion occur in a single step because: (a) Our data show that there is a substantial chemical barrier to conversion of the C-2 oxyanion of glyceraldehyde to the C-1 oxyanion of dihydroxyacetone in water.[31] Therefore, the concerted mechanism for this reaction cannot be *enforced* by the absence of a significant lifetime of the intermediate in water.[32−34] (b) There is no obvious advantage to the coupling of deprotonation of the C-2 hydroxyl by DO^- to hydride transfer in a concerted reaction mechanism.[35]

Catalysis of Isomerization with Hydride Transfer. Figure 3 shows the dependence of $(k_{iso})_{obsd}$ (s^{-1}) for isomerization of glyceraldehyde on the concentration of added Zn^{2+} at constant pD in the presence of low concentrations (40 mM) of buffers in D_2O at 25 °C (I = 1.0, KCl). The values of $(k_{iso})_{obsd}$ for these reactions are *at least* 30-fold larger than the rate constants for DO^--catalyzed isomerization at these pDs calculated using eq 8. The formation of **H-DHA** as the sole product of isomerization shows that these Zn^{2+}-catalyzed reactions proceed essentially exclusively by intramolecular transfer of hydride ion. Metal ion catalysis of related hydride transfer reactions in organic solvents,[5,7−9] and of isomerization of mannose 6-phosphate to give fructose 6-phosphate in water catalyzed by 33 mM zinc acetate at pH 6.0 and 50 °C have been reported.[10] The mechanism for the latter reaction was not investigated, but the data reported here suggest that this occurs with intramolecular transfer of hydride.

The plots of $(k_{iso})_{obsd}$ against $[Zn^{2+}]_{tot}$ in Figure 3 (data from Table S1) are approximately linear, and the slopes of these correlations were multiplied by $(1 + K_{hydrate}) = 20$ to give $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}), the apparent second-order rate constants for the zinc-catalyzed isomerization of glyceraldehyde with hydride transfer. The negative y-intercepts observed for three of these correlations are discussed below. Figure 4 shows the linear logarithmic plot of $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) against pD, which shows

Scheme 6



that the catalytic reactivity of Zn^{2+} increases with increasing $[DO^-]$. This provides strong evidence that the C-2 oxyanion of glyceraldehyde is the reactive species in both the "solvent" and the Zn^{2+}-catalyzed isomerization of glyceraldehyde with hydride transfer. The solid line in Figure 4 has a slope of 1.0 and shows the fit of the data to eq 9 derived for Scheme 3

$$\log (k_{Zn})_{hyd} = \log \left(\frac{(k_{Zn})_{hyd} K_w}{0.79} \right) + pD \qquad (9)$$

where $K_w = 10^{-14.87}$ M^2 is the ionization constant of D_2O at 25 °C[28a] and 0.79 is the apparent activity coefficient of lyoxide ion under our reaction conditions.[25b] The fit gives $(k_T)_{hyd} = 1.9 \times 10^4$ M^{-2} s^{-1} for isomerization of glyceraldehyde with formal catalysis by both DO^- and Zn^{2+} (Scheme 3), and shows that there is no significant pD-independent term for the Zn^{2+}-catalyzed reaction. The only reasonable role of zinc in catalysis of this reaction is to provide stabilization of formal negative charge at O-1 and O-2 in the transition state for hydride transfer (Scheme 6A).

Catalysis of Isomerization with Proton Transfer. The Zn^{2+}-catalyzed isomerization of glyceraldehyde in 40 mM acetate buffer at pD 5.7 proceeds exclusively with hydride transfer (Figure 3). However, competitive isomerization with proton transfer to form **D-DHA** is observed for reactions in the presence of Zn^{2+} and higher concentrations of acetate buffer at pD 5.7 (Table 1). Table 1 gives the values of $(k_{iso})_{prot}$ and $(k_{iso})_{hyd}$ (s^{-1}, Scheme 2) for isomerization with proton transfer and hydride transfer that were calculated from the values of $(k_{iso})_{obsd}$ and the fractional yields of **D-DHA** (f_{D-DHA}) and **H-DHA** (f_{H-DHA}) using eqs 10 and 11, respectively. The slopes of linear correlations of $(k_{iso})_{prot}$ and $(k_{iso})_{hyd}$ against $[Zn^{2+}]_{tot}$ (not shown) were multiplied by $(1 + K_{hydrate}) = 20$ to give the apparent second-order rate constants $(k_{Zn})_{prot}$ (M^{-1} s^{-1}) and $(k_{Zn})_{hyd}$ (M^{-1} s^{-1}) for zinc-catalyzed isomerization of glyceraldehyde with proton and hydride transfer listed in Table 1.

$$(k_{iso})_{prot} = (k_{iso})_{obsd} f_{D-DHA} \qquad (10)$$

$$(k_{iso})_{hyd} = (k_{iso})_{obsd} f_{H-DHA} \qquad (11)$$

The values of $(k_{Zn})_{hyd}$ reported in Table 1 are not significantly different from $(k_{Zn})_{hyd} = 1.7 \times 10^{-5}$ M^{-1} s^{-1} determined for Zn^{2+}-catalyzed isomerization in the presence of 40 mM acetate buffer at the same pD (Figure 3). By contrast, the values of $(k_{Zn})_{prot}$ for Zn^{2+}-catalyzed isomerization with proton transfer increase with increasing concentrations of acetate buffer (Table 1). This shows that there is a termolecular pathway for catalysis of deprotonation of glyceraldehyde by the combined action of AcO^- and Zn^{2+} catalysts. A value of $(k_T)_{prot} = 1 \times 10^{-4}$ M^{-2} s^{-1} for the third-order rate constant for this reaction (Scheme

(31) A value of $k_{hyd} \approx 0.1$ s^{-1} for unimolecular conversion of the C-2 oxyanion of glyceraldehyde to the C-1 oxyanion of dihydroxyacetone by intramolecular hydride transfer can be estimated from the relationship $k_{hyd} = (k_{DO})_{hyd} K_w/(K_a)_{O2}$ using values of $K_w = 10^{-14.87}$ [ref 28a], $(k_{DO})_{hyd} = 0.6$ M^{-1} s^{-1}, and $(K_a)_{O2} \approx 10^{-14}$ M for ionization of the O-2 hydroxyl of glyceraldehyde. The latter acidity constant was estimated by assuming that the addition of a hydroxymethyl group will lower the pK_a of 14.8 for the hydroxyl group of glycolaldehyde by ~1 unit [Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology, Physical and Chemical Data*; 3rd ed.; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, pp 305−351].

(32) Jencks, W. P. *Acc. Chem. Res.* **1976**, *9*, 425−432.
(33) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161−169.
(34) Jencks, W. P. *Chem. Soc. Rev.* **1981**, *10*, 345−375.
(35) Dewar, M. J. S. *J. Am. Chem. Soc.* **1984**, *106*, 209−219.

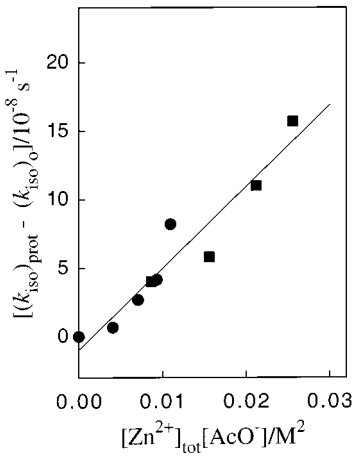


Figure 5. Dependence of the increase in the first-order rate constant for isomerization of glyceraldehyde with proton transfer $[(k_{iso})_{prot} - (k_{iso})_o]$ on the product of the concentrations of total added Zn^{2+} and free acetate anion $[Zn^{2+}]_{tot}[AcO^-]$ in D_2O at 25 °C ($I = 1.0$, KCl) (see text). The slope of the linear correlation of the data was multiplied by $(1 + K_{hydrate}) = 20$ to give $(k_T)_{prot} = 1 \times 10^{-4}$ M^{-2} s^{-1} as the third-order rate constant for isomerization of glyceraldehyde with proton transfer catalyzed by the combined action of acetate and zinc ions (Scheme 6B).

3) was determined from the slope of the plot of $[(k_{iso})_{prot} - (k_{iso})_o]$ against $[Zn^{2+}]_{tot}[AcO^-]$ shown in Figure 5 where (a) $(k_{iso})_o$ is the first-order rate constant for isomerization in the absence of added Zn^{2+}, (b) $[Zn^{2+}]_{tot}$ is the total concentration of added Zn^{2+}, and (c) $[AcO^-]$ is the concentration of free acetate anion calculated from $[buffer]_{tot}$, the solution pD, $pK_a = 5.1$ for ionization of acetic acid in D_2O, and $K_{as} = 7$ M^{-1} for formation of the $AcO^-\cdot Zn^{2+}$ complex (eq 12). This analysis assumes that free Zn^{2+} and the $AcO^-\cdot Zn^{2+}$ complex have equal *electrophilic* reactivities in catalysis of isomerization (see below).

$$AcO^- + Zn^{2+} \overset{K_{as}}{\rightleftharpoons} AcO^-\cdot Zn^{2+} \qquad (12)$$

The termolecular pathway for deprotonation of glyceraldehyde catalyzed by the combined action of zinc and acetate ions is analogous to buffer catalysis of deprotonation of acetone and other carbon acids by the combined action of the acidic and basic forms of buffer catalysts,[36] or to acetic acid-catalyzed deprotonation of acetone which proceeds by protonation of the carbonyl group followed by carbon-deprotonation by acetate ion.[4] The buffer acid, proton, and metal ion serve similar functions in these termolecular reactions in stabilization of negative charge at the carbonyl oxygen that develops in the transition state for proton transfer (Scheme 6B).

Reactive Forms of Zn^{2+}. Under our reaction conditions Zn^{2+} exists as a mixture of the hydrated cation and the mono adduct with acetate anion $AcO^-\cdot Zn^{2+}$. Because of the difficulty of monitoring these reactions by ^1H NMR and the complexity of the required kinetic analyses we have not attempted to systematically evaluate the relative electrophilic reactivity of the two different forms of zinc in catalysis of isomerization of glyceraldehyde with proton and hydride transfer. Rather, the data were evaluated to obtain *apparent* second-order rate constants k_{Zn} and third-order rate constants k_T which characterize the *bulk* reactivity of the Zn^{2+} present in solution in catalysis of isomerization.

(36) Hegarty, A. F.; Dowling, J. P.; Eustace, S. J.; McGarraghy, M. *J. Am. Chem. Soc.* **1998**, *120*, 2290−2296. Hegarty, A. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 7188−7189.

The electrophilic reactivity of the $AcO^-\cdot Zn^{2+}$ complex is expected to be lower than that of free Zn^{2+}; however, our data do not provide strong evidence for a large difference in the electrophilic reactivity of these species. The negative *y*-intercepts observed for three of the plots in Figure 3 are consistent with upward curvature for reactions in the presence of high concentrations of Zn^{2+}. The fraction of added zinc present in the [putatively] more reactive free form increases with increasing $[Zn^{2+}]_{tot}$, and it is possible that this may be reflected by an increase in the slopes of the correlations in Figure 3 at high $[Zn^{2+}]_{tot}$. By contrast, the observation that the effectiveness of zinc as a catalyst of isomerization with hydride transfer does not depend strongly on the total concentration of buffer ($(k_{Zn})_{hyd}$, Table 1) is consistent with similar electrophilic reactivities of free Zn^{2+} and the $AcO^-\cdot Zn^{2+}$ complex in catalysis of isomerization with hydride transfer. *In any event, the true rate constants for isomerization catalyzed by free zinc cannot be more than 2.5 times as large as the apparent rate constants reported here, because in all cases >40% of the total zinc was present as the free form in solution.*

The results of a theoretical examination of the mechanism of aldose−ketose isomerization show that the proton-transfer pathway is favored for the gas-phase reaction in the absence of metal ion but that the hydride transfer pathway becomes favored for reaction in the presence of a metal ion.[37] Our results show that the aldose−ketose isomerization reaction catalyzed by Zn^{2+} is substantially faster than the proton-transfer reaction catalyzed by hydroxide ion alone but that Zn^{2+} provides effective catalysis of isomerization with both proton and hydride transfer.

Zn^{2+} catalysis of hydride transfer is expected to be stronger than Zn^{2+} catalysis of proton transfer, because the metal dication interacts with a full negative charge at the transition state for the former reaction (Scheme 6A) but with only a partial negative charge at the reacting carbon acid in the transition state for the latter reaction (Scheme 6B). However, our results suggest that the difference in the stabilization of these transition states by interaction with the metal ion is not large.

Summary of Chemical Studies

Our data show that the past uncertainty in the mechanism of nonenzymatic aldose−ketose isomerization is a consequence of the following diversity of mechanistic pathways for these reactions.

(1) Solvent-catalyzed isomerization of glyceraldehyde with proton and hydride transfer in dilute aqueous alkali ([KOD] = 0.01−0.10 M) occur at similar rates (Figure 1). The similarity of rates in this solvent is fortuitous, and the hydride transfer reaction is dominant in organic solvents.[5,7−9]

(2) The relatively large barriers to isomerization with proton and hydride transfer can each be lowered by the addition of appropriate catalytic reagents.

(3) Brønsted bases provide effective catalysis of isomerization of glyceraldehyde by rate-determining deprotonation at C-2; this will normally be the only reaction detected in buffered solution at neutral pH.

(4) Zn^{2+} provides effective electrophilic catalysis of isomerization with hydride transfer (Figure 3 and Scheme 6A). This is the only reaction detected at neutral pH in the presence of low concentrations of buffer (~40 mM), and it is essentially the only pathway observed for metal ion catalysis in organic solvents.[5,7−9]

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(5) The ratio $(k_T)_{hyd}/(k_{DO})_{hyd} = 3 \times 10^4$ M^{-1} shows that 1 M Zn^{2+} results in a 30000-fold acceleration of the *spontaneous* isomerization of glyceraldehyde with hydride transfer. This corresponds to a 6 kcal/mol stabilization of the transition state for hydride transfer by Zn^{2+}.

(6) Termolecular Zn^{2+}-catalyzed isomerization with proton-transfer becomes competitive with the hydride transfer pathway for isomerization in the presence of high concentrations of Zn^{2+} and the Brønsted base acetate ion (Table 1 and Figure 5). This is due to electrophilic assistance by Zn^{2+} of general-base-catalyzed deprotonation of glyceraldehyde at C-2 (Scheme 6B).

Imperatives for Enzymatic Catalysis of Aldose−Ketose Isomerization. The process of natural selection has in many cases ensured that modern enzymes follow efficient mechanisms with optimal kinetic parameters.[12,38] The maximum enzymatic rate acceleration for a given stabilization of an enzyme-bound transition state will be obtained when the catalyst acts to stabilize the transition state for the lowest-energy reaction pathway in solution. This provides a strong imperative that enzyme catalysts follow the same reaction mechanism observed in solution. It is less likely that the mechanism for enzyme-catalyzed and solution reactions will be different, because in this case the protein catalyst must first lower the barrier to that for the *observed* solution reaction before there is net catalysis from additional stabilization of the enzyme-bound transition state. Therefore, the observation that enzyme-catalyzed isomerization reactions proceed by a proton-transfer mechanism in some cases[11,12] and with intramolecular transfer of hydride in others[13−16] suggests that isomerization by these two mechanisms proceeds with similar efficiency in water.

This prediction is confirmed by the present work. Isomerization of glyceraldehyde in alkaline D_2O results in a 40% yield of **H-DHA** from intramolecular transfer of hydride ion and a 60% yield of **D-DHA** from DO^--catalyzed deprotonation at C-2 followed by hydron transfer from D_2O to C-1 (Scheme 4). Brønsted bases catalyze isomerization solely by a proton-transfer mechanism, and Zn^{2+} provides highly effective catalysis of isomerization with hydride transfer, with $(k_T)_{hyd}/(k_{DO})_{hyd} = 3 \times 10^4$ M^{-1}. Protein catalysts have utilized the catalytic activity of these species by incorporating a Brønsted base catalyst at the active site of enzymes which catalyze aldose−ketose isomerization with proton transfer,[39] and divalent metal ions at the active site of enzymes that catalyze isomerization with hydride transfer.[40,41]

Sugar phosphates undergo efficient ($k_{cat}/K_m \approx 10^8$ M^{-1} s^{-1}) enzyme-catalyzed isomerization by a proton-transfer mechanism,[12,38] while enzymatic isomerization of simple sugars with hydride transfer is much slower ($k_{cat}/K_m \approx 10^3$ M^{-1} s^{-1}).[42] There must also be a large difference in the potential intrinsic binding energy for charged sugar phosphate dianions and neutral sugars at enzyme catalysts,[43] and it is interesting to speculate that the large potential for binding interactions between the phosphodianion group and protein catalysts has resulted in apparent perfection in catalysis of isomerization with proton transfer,[12,38] while the smaller binding energy that is available to stabilize enzyme complexes with simple sugars *must* limit the effectiveness of catalysis of their chemical reactions.

At least one sugar isomerase has evolved two metal ion binding sites.[40,41] This suggests that the focus for transition-state stabilization is the development of strong Columbic interactions between the sugar oxyanion and enzyme-bound metal cations. The low catalytic activity for sugar isomerases ($k_{cat}/K_m \approx 10^3$ M^{-1} s^{-1})[42] may represent the limit for transition-state stabilization from these interactions with enzyme-bound substrate.[43] On the other hand, the evolution of an enzyme which catalyzes isomerization of glyceraldehyde with the same efficiency as triosephosphate isomerase-catalyzed isomerization of glyceraldehyde 3-phosphate would require a similar 3×10^{10}-fold increase in k_{cat}/K_m over that observed for isomerization catalyzed by the small tertiary amine 3-quinuclidinone.[4] This would correspond to a ∼14 kcal/mol stabilization of the transition state for the hypothetical enzyme-catalyzed compared with buffer-catalyzed isomerization of glyceraldehyde by specific binding interactions between the protein and the bound ligand.[43] While it is not clear whether there is sufficient functionality at the 3-carbon substrate glyceraldehyde to allow for the development of such tight binding interactions during the course of enzymatic catalysis of isomerization to form dihydroxyacetone, such catalysis must be facilitated by incorporation of the charged phosphate dianion into the isomerization substrate.

This discussion assumes that the evolutionary time scale is sufficiently long to ensure that, through the process of natural selection, enzymes will adopt the pathway that is chemically most efficient and that it is most efficient for enzymes to stabilize the transition state that is of lowest energy in the absence of protein catalyst. While these chemically based assumptions can account for the existence of enzymes which catalyze aldose−ketose isomerization with hydride- and proton transfer, the situation is complicated by uncertainties that arise from our ignorance of the time scale for evolution of different enzymatic activities, and of the relative intrinsic *ease* for stabilization of different rate-determining transition states. For example, the difference in the catalytic activity for enzymatic catalysis of isomerization of sugar phosphates with proton transfer and of sugars with hydride transfer might reflect the longer time scale for evolution of ubiquitous glycolytic enzymes such as triosephosphate isomerase, compared with sugar isomerases, or for the greater *ease* of catalysis of isomerization with proton- compared with hydride transfer. However, we know of no evidence that the relatively simple explanation offered here to account for the existence of two different solutions to the problem of catalysis of aldose−ketose isomerization is inadequate.

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Supporting Information Available: Table S1: Observed rate constants for isomerization of glyceraldehyde in the presence of added Zn^{2+} in buffered solutions of D_2O at 25 °C and $I = 1.0$ (KCl) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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