Mechanistic Imperatives for Enzymatic Catalysis of Aldose−Ketose Isomerization: Isomerization of Glyceraldehyde in Weakly Alkaline Aqueous Solution Occurs with Intramolecular Transfer of a Hydride Ion

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It is not known whether intramolecular transfer of a hydride ion is a viable mechanism for isomerization of an α-hydroxy aldehyde to the corresponding α-hydroxy ketone in neutral and basic aqueous solutions, in view of the fast competing pathway for isomerization with proton transfer.[1] This question is of current relevance to the mechanism of action of xylose isomerase, which may catalyze aldose−ketose isomerization with intramolecular transfer of a hydride ion.[2−6] Evolution by natural selection of a catalyst which stabilizes a transition state that is of higher energy than that for the uncatalyzed reaction in water will not be favored, because the initial stabilization of such a transition state will not increase the rate of the reaction in solution. Therefore, the proton transfer mechanism observed for the general-base-catalyzed[7] and triosephosphate-isomerase-catalyzed[8] isomerization of D-glyceraldehyde 3-phosphate to dihydroxyacetone phosphate will also be strongly favored for other enzyme-catalyzed aldose−ketose isomerization reactions, unless the activation barriers for isomerization of the substrate by hydride and proton transfer in water are similar. *We report that the aldose−ketose isomerization of* D,L*-glyceraldehyde to give dihydroxyacetone in dilute alkaline solution by proton and hydride transfer proceeds at similar rates and that there is no strong mechanistic imperative for enzymatic catalysis of this isomerization by either reaction mechanism.*

Parts A and B of Figure 1 show partial ^{1}H NMR spectra of the hydroxymethylene groups of dihydroxyacetone, after 1% (deuterium-decoupled spectrum) and 4% conversion, respectively, of D,L-glyceraldehyde (D,L-GA) to dihydroxyacetone (DHA) in 0.01 M KOD in D_2O at 25 °C.[9a,10a] Figure 1C shows the partial ^{1}H NMR spectrum after 8% conversion of D,L-GA to DHA in 150 mM potassium pyrophosphate buffer in D_2O at pD 8.4. The singlet at *ca.* 4.4 ppm in each spectrum is due to the two CH_2OD groups of DHA, **1-H**, and the single CH_2OD group of monodeuteriated DHA, **1-D** (Scheme 1). The signal for the CHDOD group of **1-D** is shifted 0.024 ppm upfield from the singlet for the CH_2OD groups and appears either as a singlet in a deuterium-decoupled spectrum (Figure 1A) or as a triplet in the absence of decoupling (Figure 1B,C). The ratio of the integrated areas of the peaks for the CH_2OD and CHDOD groups of DHA in the spectrum in Figure 1C is A_{CH_2}/A_{CHD} =

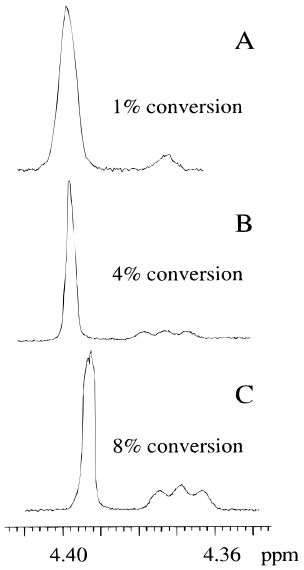
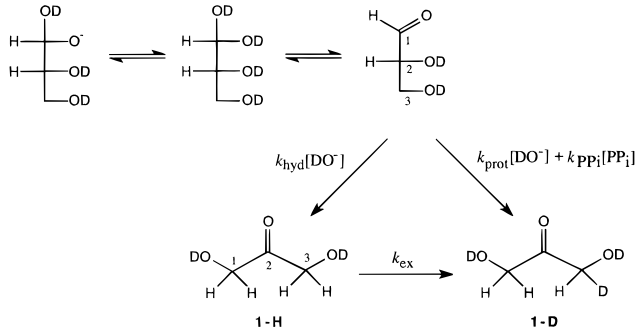


Figure 1. Representative partial 500 MHz ^{1}H NMR spectra (obtained at 25 °C in D_2O), in the region of the hydroxymethylene groups of DHA, of the reaction mixture for isomerization of D,L-GA to DHA in D_2O at 25 °C. (A,B) Isomerization in 0.01 M KOD (I = 0.10, KCl). (C) Isomerization in 150 mM potassium pyrophosphate buffer at pD 8.4. The singlets at *ca.* 4.4 ppm are due to the CH_2OD groups of DHA and monodeuteriated DHA. The upfield-shifted singlet in the deuterium-decoupled spectrum (A) and the triplets in the absence of decoupling (B and C), are due to the CHDOD group of monodeuteriated DHA.

Scheme 1



(1) (a) The small amount of intramolecular transfer of tritium observed during the *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). However, the interpretation of these data is clouded by uncertainties about the mechanism and possible contribution of a much faster aerobic pathway for intramolecular transfer of tritium. 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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(9) (a) Isomerization of D,L-GA in a volume of 10 mL was initiated by making a 30-fold dilution of a 30 mM solution of substrate in D_2O (99.9% from Cambridge Isotope Laboratories) into the reaction mixture. The reactions in dilute alkaline solution were quenched at timed intervals by adjusting the solution to pD ≈ 5 using 1.0 M DCl. The volume of the solution was then reduced to *ca.* 1 mL by evaporation of the solvent under reduced pressure (T ≤ 30 °C). A similar procedure was used to follow isomerization in 150 mM potassium pyrophosphate buffer, except that the final concentration of substrate was 1.5 mM, and the reactions were quenched by adjusting the pD to between 2 and 3. Control experiments showed that there was no isomerization of D,L-GA or exchange of deuterium from solvent into DHA during concentration of the reaction mixture. (b) The exchange of deuterium from D_2O into the hydroxymethylene groups of DHA was monitored by ^{1}H NMR,[15,16] and the observed first-order rate constants for deuterium exchange were determined by treating the data as described in ref 14.

(10) (a) ^{1}H NMR spectra of the reaction mixtures for the isomerization of D,L-GA were obtained in D_2O at 25 °C using a Varian Unity INOVA-500 spectrometer. ^{1}H NMR spectra of the reaction mixtures for the exchange of deuterium from solvent into DHA were obtained in D_2O at 25 °C using Varian VXR-400S or INOVA-500 spectrometers. Chemical shifts are reported relative to HOD at 4.65 ppm. (b) These values were calculated by correcting the observed ratios of 5.7 (Figure 1A) and 4.1 (Figure 1B) for the presence of 0.15% of dihydroxacetone in our preparation of D,L GA.

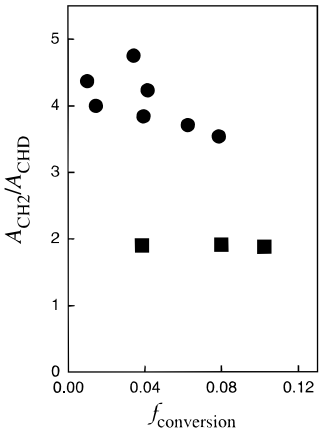

Figure 2. The change in the ratio of the integrated areas of the peaks for the CH_2OD and CHDOD groups of the product, A_{CH_2}/A_{CHD}, with increasing extents of conversion of D,L-GA to DHA in D_2O at 25 °C. (●) Isomerization in 0.01 M KOD ($I = 0.10$, KCl). (■) Isomerization in 150 mM potassium pyrophosphate buffer at pD 8.4.

1.9, which is consistent with isomerization of D,L-GA to give DHA which contains one CHDOD group and one CH_2OD group (**1-D**), as is required for isomerization of the substrate by a proton transfer mechanism catalyzed by pyrophosphate ion (k_{PP_i}-[PP_i], Scheme 1). By contrast, the ratios $A_{CH_2}/A_{CHD} = 4.4$ and 3.8 determined from the spectra in parts A and B of Figure 1,[10b] respectively, show that isomerization of D,L-GA in 0.01 M KOD results in the incorporation of only 0.63 and 0.69, respectively, mole equivalent of deuterium from solvent into DHA. Therefore, this isomerization reaction proceeds with *partial* intramolecular transfer of hydrogen to give a mixture of **1-H** and **1-D** (k_{hyd}[DO^-], Scheme 1). This hydrogen must be transferred from C-2 of glyceraldehyde to C-1 of DHA in the form of a hydride ion, because the molecule of DOH formed by deprotonation of the substrate by deuteroxide ion undergoes essentially complete equilibration with DOD from bulk solvent before there is significant reprotonation of the enediolate intermediate to give **1-H**.[11]

Figure 2 (●) shows that there are significant decreases in the ratio A_{CH_2}/A_{CHD} determined for the isomerization of D,L-GA in 0.01 M KOD with increasing extents of conversion of D,L-GA to DHA. These decreases are due to the base-catalyzed exchange of deuterium from solvent into **1-H** and **1-D** (k_{ex}, Scheme 1). The observed first-order rate constant for isomerization, $(k_{iso})_{obsd} = 3.7 \times 10^{-4}$ s^{-1}, was determined from the initial velocity of conversion of the first 4% of D,L-GA to **1-H** and **1-D** in 0.01 M KOD in D_2O ($I = 0.10$, KCl) at 25 °C,[12] and the rate constant for exchange of deuterium from solvent into DHA under the same conditions was determined to be $k_{ex} = 1.7 \times 10^{-3}$ s^{-1}.[9b] These rate constants show that the exchange of deuterium into **1-H** is not fast enough to account for the observed formation of **1-D** during the early stages of the isomerization reaction. For example, only 4% of **1-H** undergoes exchange with deuterium during the time required for isomerization of 1% of D,L-GA, but the yield of **1-D** from the first 1% of this isomerization is 63%.

The decrease in A_{CH_2}/A_{CHD} with increasing extents of isomerization of D,L-GA is slower for reaction in the presence of 150 mM potassium pyrophosphate at pD 8.4 (Figure 2, ■) than for reaction in dilute alkali (0.01 M KOD), because this change in conditions causes a decrease in the rate constant for washout of hydrogen from DHA ($k_{ex} = 2.7 \times 10^{-5}$ s^{-1})[9b] relative to isomerization ($(k_{iso})_{obsd} = 1.6 \times 10^{-5}$ s^{-1}).[12] The data in Figure 2 (●) show that the products of isomerization of D,L-GA with intramolecular transfer of hydride can be easily detected only during the early stages of the reaction, because of the competing isomerization with proton transfer and exchange of deuterium from solvent into DHA. Therefore, the failure to detect intramolecular transfer of tritium during the hydroxide-ion-catalyzed isomerization of [2-^{3}H]-D-glucose to D-fructose[1] may have been due to a relatively low level of intramolecular transfer of tritium, and the subsequent washout of tritium from the reaction product.

The ratio of the integrated areas of the peaks for the CH_2OD and CHDOD groups of the *initial products* of the isomerization reaction in 0.01 M KOD is $A_{CH_2}/A_{CHD} = 4.5 \pm 0.5$.[13] This was substituted into eq 1 to give [**1-H**]/[**1-D**] = $(k_{hyd})_{obsd}/(k_{prot})_{obsd} = 0.63 \pm 0.1$ for the ratio of the observed first-order rate constants in 0.01 M KOD for isomerization of D,L-GA by hydride transfer to give **1-H** and by proton transfer to give **1-D**.

$$[\mathbf{1\text{-}H}]/[\mathbf{1\text{-}D}] = (A_{CH_2} - 2A_{CHD})/(4A_{CHD}) \qquad (1)$$

In conclusion, we have shown that the rate constants for isomerization of D,L-GA in a dilute alkaline solution by hydride and proton transfer mechanisms are closely matched, so that there is no obvious advantage to enzymatic catalysis of aldose−ketose isomerization by either pathway. These results provide a solid chemical precedent for the proposal that enzyme-catalyzed aldose−ketose isomerization reactions may proceed with intramolecular transfer of a hydride ion.[2−6]

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(11) This argument is developed in greater detail in ref 16.

(12) The progress of isomerization of D,L-GA was determined by comparison of the integrated areas of the peaks for the hydroxymethylene protons of DHA and for the C-1 proton of the hydrate of D,L-GA, with appropriate corrections for the differences in the extent of hydration of DHA (16% hydrate) and D,L-GA (95% hydrate).

(13) This was estimated by a short linear extrapolation to zero time of the values for A_{CH_2}/A_{CHD} determined over the first 4% reaction. Multiple determinations of A_{CH_2}/A_{CHD} during the first 1%−4% of the isomerization reaction in dilute alkaline solution showed that this ratio is reproducible to within ±10%.

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