Reaction of triosephosphate isomerase with L-glyceraldehyde 3-phosphate and triose 1,2-enediol 3-phosphate

ARTICLE *in* BIOCHEMISTRY · MARCH 1985

Impact Factor: 3.02 · DOI: 10.1021/bi00325a021 · Source: PubMed

1 AUTHOR:

John P. Richard
University at Buffalo, The State University of …

219 PUBLICATIONS **6,939** CITATIONS

SEE PROFILE

Reaction of Triosephosphate Isomerase with L-Glyceraldehyde 3-Phosphate and Triose 1,2-Enediol 3-Phosphate†

John P. Richard*

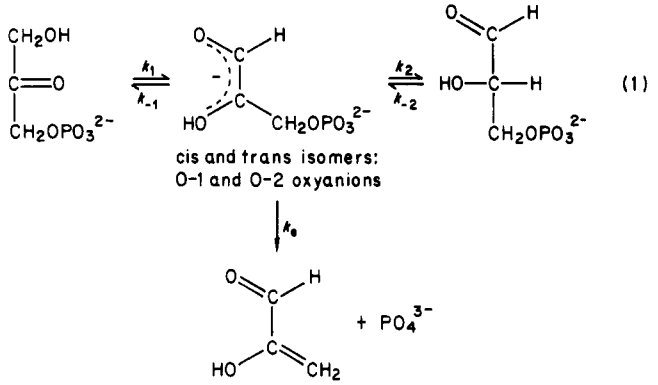
Institute of Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111
Received June 4, 1984

ABSTRACT: Triosephosphate isomerase catalyzes the isomerization and/or racemization reactions of L-glyceraldehyde 3-phosphate (LGAP), the enantiomer of the physiological substrate. The reaction is inhibited by the active site directed reagent glycidol phosphate. The amount of protonation product formation catalyzed by a fixed enzyme concentration is nearly independent of increasing steady-state concentrations of triose 1,2-enediol 3-phosphate caused by buffer catalysis of LGAP deprotonation. Therefore, enzymatic protonation of the enediol or enediolate, which could account for the observed enzymatic catalysis of LGAP isomerization and/or racemization, is at best a minor reaction. Instead LGAP reacts directly at the enzyme active site. Triosephosphate isomerase catalysis of the protonation of triose 1,2-enediol 3-phosphate was expected because of the strong evidence supporting an enediol reaction intermediate for the overall reaction catalyzed by isomerase. The most reasonable explanation for the failure to observe enzymatic protonation is that in solution the enediol undergoes β elimination of phosphate ($t_{1/2}$ is estimated to be 10^{-6} s) faster than it can diffuse to and form a complex with isomerase.

Considerable experimental evidence has been accumulated supporting an enediol(ate) intermediate for the aldose–ketose isomerization reaction catalyzed by triosephosphate isomerase.

(1) The enzyme catalyzes the rapid solvent exchange reaction of a proton α to the carbonyl group of dihydroxyacetone phosphate (DHAP) or D-glyceraldehyde 3-phosphate (DGAP) (Rieder & Rose, 1959). This result rules out a direct hydride transfer mechanism.

(2) Triosephosphate isomerase catalyzes both the isomerization and elimination reactions of triose phosphates (Iyengar & Rose, 1981). This is consistent with an enediolate intermediate, because in solution the enediolate partitions between protonation and β elimination of phosphate (eq 1) (Richard,



(1)

1984; Hall & Knowles, 1975; Bonsignore et al., 1973).

(3) The nonenzymatic isomerization reaction in water is through an enediolate intermediate (Richard, 1984). The same mechanism will be favored for the enzymatic reaction because it requires a smaller enzymic rate acceleration than other mechanisms.

In order to study the reaction of the enediol(ate) intermediate with isomerase, this species has been generated nonenzymatically by deprotonation of L-glyceraldehyde 3-phosphate (LGAP) (the enantiomer of the enzymatic substrate). Triosephosphate isomerase catalysis of the second half of the isomerization reaction, i.e., enediol(ate) protonation, will divert the reaction of the intermediate from the elimination pathway to the isomerization and/or racemization pathways (eq 1). The net increase in the fraction of the intermediate partitioning to protonation products will be manifested experimentally as enzymatic catalysis of the isomerization and/or racemization reactions of LGAP.

The results reported here show that triosephosphate isomerase catalyzes the isomerization and/or racemization reactions of LGAP. However, it is also shown that the mechanism for this reaction requires that LGAP react directly with the enzyme in a manner comparable to the reaction of DGAP. Enzymatic protonation of the enediol(ate) generated from LGAP is at best a minor reaction.

Materials and Methods

Most of the materials and methods used here have been reported previously (Richard, 1984). Rabbit muscle triosephosphate isomerase (9000 units/mg) was from Calbiochem-Behring. The enzyme was shown to be nearly homogeneous by sodium dodecyl sulfate (SDS) gel electrophoresis but contained a trace impurity with a molecular weight of ~15 000. The enzyme subunit concentration was calculated from the absorbance at 280 nm and an extinction coefficient of 3.1×10^4 M^{-1} cm^{-1} (McVittie et al., 1972). Triosephosphate isomerase was assayed by coupling DGAP isomerization to NADH oxidation with α-glycerol-phosphate dehydrogenase. Triosephosphate isomerase was inactivated with DL-glycidol phosphate (Rose & O'Connell, 1969; Schray et al., 1973) by the following procedure. Two milligrams of the barium salt of DL-glycidol phosphate was converted to the free acid by treatment with Dowex 50 and then incubated at 37 °C with 1.5 mg of triosephosphate isomerase in 0.3 mL of 0.1 M triethanolamine hydrochloride (pH 7.9) for 24 h. At this time the enzyme retained 0.3% of its original activity.

The isomerization and racemization reactions of LGAP in the presence of triosephosphate isomerase were followed by

† This work was supported by U.S. Public Health Service Grants GM-20940, CA-06927, and RR-05539 and also supported by an appropriation from the Commonwealth of Pennsylvania.

* Address correspondence to this author at the University Chemical Laboratories, Cambridge University, Cambridge, England CB2 1EW.

0006-2960/85/0424-0949$01.50/0

coupling the formation of DHAP to NADH oxidation with α-glycerol-phosphate dehydrogenase (Richard, 1984). Reaction mixtures of increasing triosephosphate isomerase concentration were prepared by replacing a stock 0.1 M triethanolamine hydrochloride buffer solution with triosephosphate isomerase which had been dialyzed against the *same* stock solution. Reactions (1.0 mL final volume) were initiated by the addition of LGAP (as a 0.2 M solution at pH 7), NADH (enough of a 0.1 M solution to give a final absorbance of ~0.6 at 340 nm), and 10 μL (~5 mg/mL) of α-glycerol-phosphate dehydrogenase. The initial velocity of NADH oxidation was determined for the first 2–10 min of reaction, and $k_{iso+rac}$ values were calculated from eq 2 ($[LGAP]_0$ is the initial concentration of LGAP). It was shown in control

$$k_{iso+rac} = \frac{d[NADH]/dt}{[LGAP]_0} \quad (2)$$

experiments that there was no oxidation of NADH in the absence of α-glycerol-phosphate dehydrogenase and that $k_{iso+rac}$ was constant for a 2-fold increasse in α-glycerol-phosphate dehydrogenase concentration. Values of $k_{iso+rac}$ were reproducible to ±10%.

The rate constants for the uncatalyzed and the buffer-catalyzed elimination reactions of [^{32}P]LGAP were measured as described previously (Richard, 1984). The reaction in the presence of triosephosphate isomerase was in a volume of 0.1 mL and contained 2 μL of α-glycerol-phosphate dehydrogenase, 2 mM [^{32}P]LGAP (5×10^7 cpm/μmol), and 2 mM NADH. Aliquots of 4 μL were withdrawn and monitored for decreasing organic phosphate (Richard, 1984).

Results

The presence of 10 mM LGAP had no effect on the observed rate of DHAP formation by the triosephosphate isomerase catalyzed reaction of 1 mM DGAP [the DGAP K_m is 0.3 mM (Reynolds et al., 1971)]. However, when the observed reaction velocity was corrected for DHAP formation by the *nonenzymatic* isomerization of LGAP (Richard, 1984), it was found that isomerase was inhibited 10% by 10 mM LGAP.

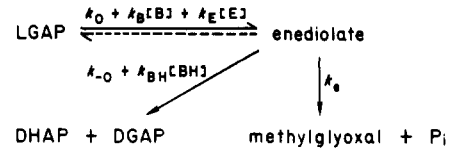
Figure 1 shows the dependence of the observed rate constants for the formation of DHAP and/or DGAP from LGAP, $k_{iso+rac}$, on the concentration of triosephosphate isomerase for reaction at 37 °C in 0.1 M triethanolamine hydrochloride (pH 7.9) and 8 mM LGAP. The slope of this plot, k_{obsd}, is 0.23 M^{-1} s^{-1}. Enzymatic catalysis occurs at the active site, because no activity is observed for glycidol phosphate inactivated enzyme (open circle in Figure 1). Previous work in this laboratory was at ionic strength of 1.0 (KCl) (Richard, 1984). Large KCl concentrations were not used here because triosephosphate isomerase is inhibited 10-fold by 0.9 M KCl.

Data for enzymatic catalysis of LGAP reaction at 0.0, 0.04, and 0.08 M quinuclidinone buffer (pH 7.8) and 2 mM LGAP are given in Figure 2. In a control experiment it was shown that the activity of triosephosphate isomerase remains constant (≤5% decrease) as the quinuclidinone buffer concentration is increased from 0 to 0.08 M.

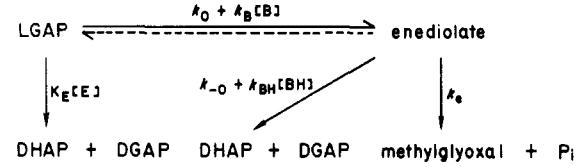
The rate constants k_{elim} for the elimination reaction of LGAP to give methylglyoxal and phosphate (see eq 1) under the conditions used in Figure 2 are respectively the following at 0.00, 0.04, and 0.08 M quinuclidinone buffer: 1.15×10^{-4} s^{-1}, 1.82×10^{-4} s^{-1}, and 2.55×10^{-4} s^{-1}.

It was previously reported (Iyengar & Rose, 1983) that 2 μM triosephosphate isomerase causes a 60% reduction in the amount of inorganic phosphate formed from LGAP. This experiment was repeated, and it was found that when the

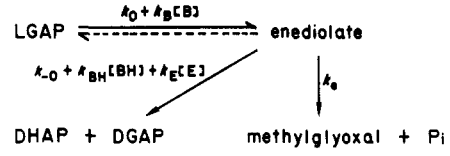
Scheme I



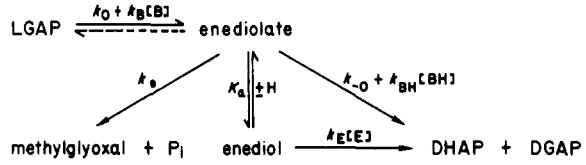
Scheme II



Scheme III



Scheme IV



elimination reaction of LGAP (2 mM) is carried out at pH 7.9 (0.1 M triethanolamine hydrochloride) and 37 °C in the presence of α-glycerol-phosphate dehydrogenase and NADH to trap isomerization products, the amount of P_i formation decreased only 10% and the rate constant k_{elim} for the LGAP elimination reaction remained constant [1.1×10^{-4} s^{-1} (±5%)] as the isomerase concentration was increased from 0 to 20 μM.[1] There is good agreement between the increase in isomerization and/or racemization products (6%, calculated from Figure 2) and the decrease in elimination products (10%) observed as the isomerase concentration is increased from 0 to 20 μM. Dr. Irwin Rose has also reexamined his previously published work and has obtained results in agreement with those reported here (Rose, 1984a).

Discussion

Mechanism for Enzymatic Catalysis of LGAP Reaction. Figures 1 and 2 show that triosephosphate isomerase catalyzes the formation of DHAP and/or DGAP from LGAP. Catalysis is only observed at high isomerase concentrations: 10^{-5} M isomerase corresponds to 2000 units/mL for enyzmatic catalysis of DGAP isomerization. In previous work it was shown that in solution LGAP undergoes essentially irreversible uncatalyzed deprotonation to give an enediolate which partitions between fast β elimination of phosphate and ≥100 times slower uncatalyzed protonation to give isomerization (DHAP) or racemization (DGAP) products (see eq 1) (Richard, 1984). It was also shown that LGAP deprotonation is strongly general base catalyzed, that enediolate protonation is general acid catalyzed, but that phosphate expulsion from the enediolate

[1] An attempt to measure k_{elim} at ≥50 μM isomerase was unsuccessful, because under these conditions the reaction did not give a stable end point (~10–20-h reaction time). The slow end point drift may have been due to α-glycerol phosphate hydrolysis by a small amount of phosphatase contaminant in the triosephosphate isomerase.

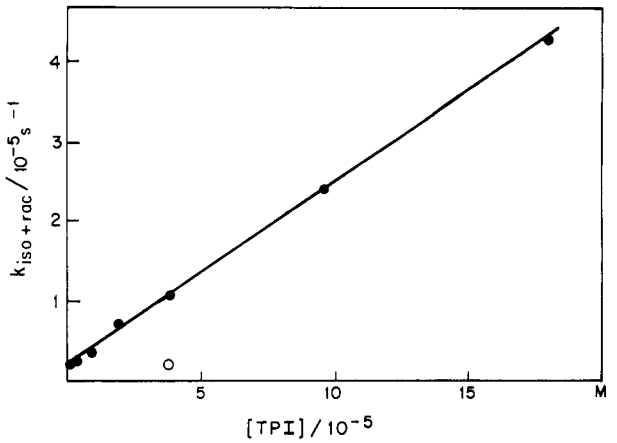


FIGURE 1: Triosephosphate isomerase catalysis of the reaction of 8 mM LGAP at 37 °C and pH 7.9 (0.1 M triethanolamine hydrochloride). The open circle is for glycidol phosphate inactivated isomerase.

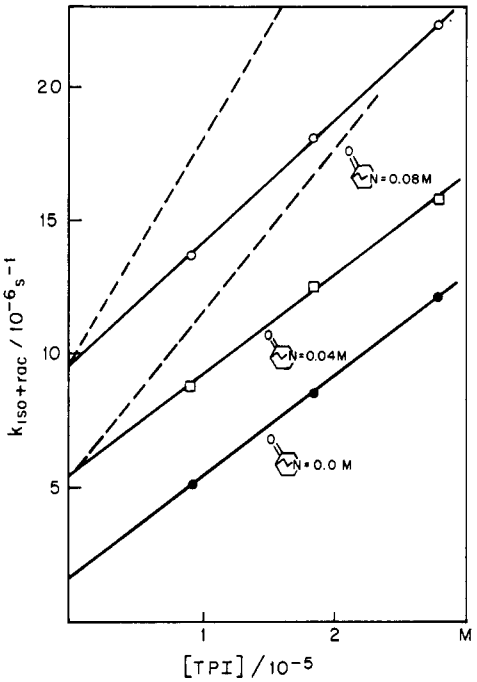


FIGURE 2: Triosephosphate isomerase catalysis of the reaction of 2 mM LGAP at 37 °C and pH 7.8 (triethanolamine) in the presence of 0 (●), 0.04 (□), and 0.08 M (○) quinuclidinone buffer. The reaction mixtures were prepared by replacing 0.1 M triethanolamine hydrochloride with 0.1 M quinuclidinone hydrochloride. The solid lines are for the calculated experimental slopes, and the dashed lines are for theoretical slopes calculated in Table I (see text) for the mechanisms given in Schemes III and IV.

is not buffer catalyzed (Richard, 1984). These observations are used as the framework for the four possible mechanisms for enzymatic catalysis of LGAP reaction given in Schemes I–IV. In Schemes I and II the enzyme reacts directly with LGAP, to catalyze either enediolate formation (Scheme I) or the overall reaction (Scheme II). In Schemes III and IV the enzyme reacts with an intermediate generated by nonenzymatic deprotonation of LGAP, either the enediolate (Scheme III) or the enediol (Scheme IV). In all these schemes a broken arrow has been drawn for enediolate return to LGAP, because LGAP deprotonation is essentially irreversible (Richard, 1984).

In Scheme I triosephosphate isomerase acts to increase the rate of enediolate formation. Enzymatic catalysis of enediolate formation must lead to the same proportional increase in the observed rate constants for the appearance of all products formed by partitioning of the enediolate. Therefore, the observation that k_{elim} remains constant while $k_{iso+rac}$ increases 4–7-fold (Figures 1 and 2) as the isomerase concentration is increased from 0 to 20 μM rules out the mechanism in Scheme I.

Table I: Buffer Dependence of Triosephosphate Isomerase Catalysis of L-Glyceraldehyde 3-Phosphate Reaction[a]

$\left[\text{quinuclidinone}\right]_T$ (M)[b]	k_{elim} ($\times 10^{-4}$ s^{-1})[c]	$1 + k_B[B]/k_0$[d]	k_{obsd} (M^{-1} s^{-1})[e]	$k_{obsd}/(k_{obsd})_0$[f]
0.00	1.15	1.00	0.38	1.00
0.04	1.82	1.58	0.37	0.97
0.08	2.55	2.22	0.46	1.21

[a] At pH 7.8, 37 °C. [b] The total buffer concentration. [c] The observed rate constant for the elimination reaction of LGAP; $k_{elim} = k_0 + k_B[B]$ (Richard, 1984). [d] The increase in the slopes of Figure 2 required if the enzymatic mechanism is that given in Schemes III or IV (see eq 9). The values are calculated from the increasing k_{elim} values (see footnote c). [e] The slopes of the lines in Figure 2. [f] The k_{obsd} at the given base concentration divided by k_{obsd} at zero base.

The dependence of $k_{iso+rac}$ on buffer and enzyme concentration for Schemes II–IV is given by eq 3–5, respectively.

$$k_{iso+rac} = k_E[E] + [(k_0 + k_B[B])(k_{-0} + k_{BH}[BH])]/k_e \quad (3)$$

$$k_{iso+rac} = f_c[[(k_0 + k_B[B]) \times (k_{-0} + k_{BH}[BH] + k_E[E])]/k_e] + (1 - f_c)(A) \quad (4)$$

$$k_{iso+rac} = [(k_0 + k_B[B])/k_e]\,(k_{-0} + k_{BH}[BH] + f_c k_E[E][H]/K_a) \quad (5)$$

These equations are derived by assuming a steady-state enediolate concentration, with the simplification that $k_e \sim k_e + k_{-0} + k_{BH}[BH] + k_E[E]$. This simplification is made because at ≤0.2 M quinuclidinone buffer concentrations enediolate partitioning to elimination products (k_e) is very much faster than partitioning to isomerization or racemization products ($k_{-0} + k_{BH}[BH] + k_E[E]$) (Richard, 1984). The term f_c in eq 4 and 5 is the fraction of enediolate present as the geometric isomer which is the substrate for enzymatic reaction (cis or trans) (Rieder & Rose, 1959; Rose, 1962); it corrects for the different concentrations of the reactive intermediate in the nonenzymatic reaction (total enediolate) and the enzymatic reaction (single geometric isomer). The term A in eq 4 contains a collection of rate constants that are independent of enzyme concentration.

The velocity of the reaction between isomerase and the intermediate will be first order in isomerase concentration because the enzyme (10^{-6}–10^{-4} M, Figures 1 and 2) is present in great excess over the enediol and enediolate. The steady-state enediol concentration is equal to

$$[(k_0 + k_B[B])[H]/K_a k_e][LGAP] \sim 10^{-9}\ M$$

where $k_0 + k_B[B] \leq 2.55 \times 10^{-4}$ s^{-1} (Table I), [LGAP] ≤ 0.008 M, [H] = $10^{-7.9}$ M, $K_a \sim 10^{-10.9}$ M (Chiang et al., 1984), and $k_e \sim 5 \times 10^5$ s^{-1} (see the following section). The steady-state enediolate concentration will be smaller still because the enediol [$pK_a \sim 10.9$ (Chiang et al., 1984)] is largely protonated at pH 7.9.

The dependence of the observed second-order rate constants for isomerase reaction, $k_{obsd} = d(k_{iso+rac})/d[E]$, on buffer general base concentration are given for Schemes II–IV by eq 6–8, respectively. The normalized dependence of k_{obsd} on general base concentration for Schemes III and IV (k_{obsd} at a given base concentration divided by k_{obsd} at zero base) is given by eq 9.

$$k_{obsd} = k_E \quad (6)$$

$$k_{obsd} = f_c k_E(k_0 + k_B[B])/k_e \quad (7)$$

$$k_{obsd} = f_c k_E[H](k_0 + k_B(B])/K_a k_e \quad (8)$$

$$k_{obsd}/(k_{obsd})_0 = (k_0 + k[B])/k_0 \quad (9)$$

The ratio $k_{obsd}/(k_{obsd})_0$ in eq 9 is also equal to the ratio of the observed LGAP elimination rate constants (k_{elim}) at the two buffer concentrations, since k_{elim} is essentially equal to the rate constant for substrate deprotonation: $k_0 + k_B[B]$ (Richard, 1984).

Schemes III (eq 7) and IV (eq 8) require a first-order dependence of k_{obsd} on base concentration because the effect of general base catalysis is to increase the steady-state concentration of the intermediate which reacts with isomerase, either the enediolate (Scheme III) or the enediol (Scheme IV). Scheme II (eq 6) predicts a zero-order dependence of k_{obsd} on [B], because here the reactions of the general base and enzyme are with different species along separate pathways.

Data for the reaction of LGAP at increasing quinuclidinone buffer concentrations are summarized in Table I. The observed second-order rate constants for the enzyme-catalyzed reaction, k_{obsd}, are nearly independent of buffer concentration, whereas the largest required increase in k_{obsd} for Schemes III and IV, calculated from the increase in k_{elim} (see above), is 120%. The dashed lines in Figure 2 show the increase in k_{obsd} required for Scheme III or IV.

The 20% increase in k_{obsd} at 0.08 M quinuclidinone is just within the estimated experimental error of ±10%; it is consistent with a >80/<20 ratio for enzyme-catalyzed isomerization through direct reaction (Scheme II) and trapping (Scheme III or IV). Therefore, it is concluded that enediol(ate) protonation by triosephosphate isomerase is at best a minor pathway for the enzyme-catalyzed reaction of LGAP.

Why Is Reaction of Isomerase with the Enediol(ate) Not Observed? There is considerable evidence supporting an enediol(ate) intermediate for the enzymatic reaction catalyzed by triosephosphate isomerase (see the introduction). If the enediol(ate) is an intermediate in the overall reaction, then isomerase must catalyze intermediate protonation to give DHAP and DGAP. Therefore, it is significant that there is little or no protonation by isomerase of the enediol(ate) formed by nonenzymatic deprotonation of LGAP. There are at least three explanations for the failure to observe protonation. They are listed in what this author considers to be their order of increasing probability.

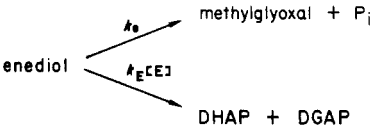
(1) The enediol(ate) is not an intermediate of the isomerase-catalyzed reaction. This is unlikely because of the strong evidence supporting an enediol(ate) intermediate (see the introduction).

(2) The intermediate formed by nonenzymatic deprotonation of LGAP has the opposite geometric configuration (cis or trans) of the triosephosphate isomerase intermediate. An upper limit for the isomer ratio consistent with the experimental results can be set as follows. Fast enzymatic trapping of a single geometric isomer and slower direct isomerization of LGAP would lead to a biphasic plot in Figure 1. The deviation of the extrapolated y intercept for the line through values at high enzyme concentrations (direct reaction) from the k_{iso} value obtained in the absence of isomerase would be related to the fraction of the active geometric isomer protonated by isomerase according to eq 10, where f_c is the

$$f_c = k_{dev}/k_0 \quad (10)$$

fraction of the enzymic isomer, k_{dev} the above experimental deviation, and k_0 the limiting rate constant for $k_{iso+rac}$ which would be observed if nonenzymatic deprotonation gave only the enzymic isomer (k_0, the rate constant for LGAP deprotonation, is limiting since for a trapping mechanism the rate of product formation cannot be faster than the rate of LGAP deprotonation). The k_{dev} value for Figure 1 is zero. In previous work at ionic strength of 1.0 (KCl), a k_{dev}/k_0 value of 1.5×10^{-7} s^{-1}/8.5×10^{-5} s^{-1} = 0.0018 was observed[2] (Richard, 1984). The small k_{dev} value was attributed to the isomerase reaction with DGAP which was formed by *nonenzymatic* enediolate protonation at C-2, because a rate constant of 1.5×10^{-7} s^{-1} for nonenzymatic LGAP racemization is consistent with rate and equilibrium data for the reaction of triose phosphates (Richard, 1984). Therefore, k_{dev} is $<1.5 \times 10^{-7}$ s^{-1} and f_c is <0.0018 at 0.9 M KCl and at least as small at 0 M KCl.

Scheme V



While a large preference for nonenzymatic deprotonation to give a single geometric isomer has not been ruled out experimentally, it is not regarded as a likely possibility. The preferential formation of a single enediolate isomer could be explained by a large difference in the ground-state energies of the conformers of LGAP, or of the cis and trans enediols, which is relieved in the transition state for uncatalyzed[3] enediolate deprotonation. However, there are good arguments which suggest that the energies of these species are similar. An examination of space-filling molecular models does not suggest that there are significant differences in intramolecular steric interactions for the syn and anti configurations of the C-1 aldehyde and C-2 hydroxyl (which upon deprotonation give the cis and trans enediols, respectively) either in the ground state or in the transition state for uncatalyzed[3] LGAP deprotonation. Similar stabilities are also expected for the cis and trans enediols because steric interactions between the double bond substituents are no larger than interactions which lead to equilibrium constants of between 0.10 and 1.0 for trans $\rightleftarrows$ cis isomerization of simple alkenes (Hine, 1975), and resonance interactions between the double bond and its substituents, which can sometimes lead to smaller equilibrium constants (Hine, 1975), are not important.

(3) The β elimination of phosphate from the enediol is faster than enzymatic protonation of the enediol. This corresponds to $k_e > k_E[E]$ for Scheme V. The value for k_e may be estimated by comparison of the reactions of the enols of acetone and dihydroxyacetone phosphate. A value of 50 s^{-1} (25 °C) for acetone enol protonation at pH 7.9 is calculated from the rate equation for protonation at pH 10–12 (Chiang et al., 1984). The same value is used for the water protonation of the enediol at pH 7.9 and 37 °C because of the offsetting corrections required for the different reaction temperatures and the slightly greater thermodynamic stability estimated for the C-3 DHAP carbanion [p$K_a \simeq 18$ (Richard, 1984)] compared to the acetone carbanion [pK_a = 19.2 (Chiang et al., 1984)]. In both cases the primary pathway for enol reaction

[2] The k_{obsd} value at ionic strength of 1.0 (KCl) [calculated from data in Richard (1984)] is about 10 times smaller than the k_{obsd} values reported here due to KCl inhibition of isomerase. The small k_{dev} value was more easily determined from the plot with the shallower slope.

[3] At pH 7.9 the mechanism for the uncatalyzed deprotonation of LGAP is through intramolecular substrate deprotonation by the phosphate dianion (Richard, 1984).

is protonation of the small pool of the highly reactive enolate by water. The rate constant for enediol phosphate protonation is faster because of intramolecular protonation of the enediolate by the phosphate monoanion. Intramolecular DHAP deprotonation at pH 7.9 is 100 times faster than hydroxide deprotonation (Richard, 1984), and there must also be a 100-fold acceleration of the protonation reaction, because the added intramolecular pathway cannot change the equilibrium constant for substrate deprotonation. The k_e value is increased further because the rate of enediol breakdown to inorganic phosphate is 100 times[4] faster than the rate of the intramolecular protonation reaction (Richard, 1984). This gives an estimated k_e value of $50\ s^{-1} \times 10^2 \times 10^2 = 5 \times 10^5\ s^{-1}$. The value for k_E will be equal to or less than the maximum rate constant for diffusional binding to the enzyme, $10^9\ M^{-1}\ s^{-1}$ (Hammes & Schimmel, 1970). Combination of k_E with the largest enzyme concentration used in this study gives a maximum value of $k_E[E] \leq (10^9\ M^{-1}\ s^{-1})\ (1.5 \times 10^{-4}\ M) \leq 1.5 \times 10^5\ s^{-1}$ for enzymatic protonation of the enediol. This is smaller than the estimated elimination rate constant k_e. Therefore, it is predicted that 1.5×10^{-4} M isomerase subunits will not trap a large fraction of the enediol, because the lifetime for the enediol in water is not long enough to allow for diffusional encounter with isomerase.

It is well recognized that triosephosphate isomerase must act to decrease the rate of enediol breakdown by β elimination of phosphate, if the species is an intermediate of the enzymatic reaction (Iyengar & Rose, 1983; Alber et al., 1981).

The facile breakdown by β elimination of phosphate will make the direct study of the chemical and enzymological reactions of the enediol of DHAP and GAP much more difficult than the study of the reactions of the enol of pyruvate. The latter compound, which is also an important enzymatic intermediate (Rose, 1982), has a half-time for deuteration in D_2O at pD 6.4 and 20 °C of 3.6 min (Kuo et al., 1979).

The results reported here are inconsistent with the previously reported enediol phosphate lifetime of 10^{-2} s and the reported 50% trapping of the enediol phosphate by 6×10^{-8} M isomerase subunits (Iyengar & Rose, 1981). The experiments from which these values were calculated have been found to be irreproducible (Rose, 1984b).

Direct Reaction of LGAP. A two step mechanism for direct enzymatic catalysis of the isomerization and/or racemization reactions of LGAP is written in Scheme VI.

Scheme VI

$$E + LGAP \underset{}{\overset{K_d}{\rightleftharpoons}} E{\cdot}LGAP \xrightarrow{k_{cat}} E + DGAP + DHAP$$

$$k_{obsd} = \frac{k_{cat}}{[LGAP] + K_d} \qquad (11)$$

Equation 11 gives the relationship between the observed second-order rate constant k_{obsd} calculated from the slope of the plots of Figures 1 and 2 (no quinuclidinone) and the kinetic parameters in Scheme VI. The k_{obsd} values at 2 (0.38 $M^{-1}\ s^{-1}$) and 8 mM (0.23 $M^{-1}\ s^{-1}$) LGAP differ by less than a factor of 2, showing that the enzyme is below saturation at 2 mM LGAP. *Approximate* values of $K_d = 7$ mM and $k_{cat} = 3.5 \times 10^{-3}\ s^{-1}$ can be calculated from a double-reciprocal form of eq 11 and the k_{obsd} values at 2 and 8 mM LGAP.[5] A comparison with the steady-state kinetic parameters for reaction of DGAP (Knowles & Albery, 1977) shows an approximate decrease of 10^6 and 10^9, respectively, in the first-order rate constant k_{cat} and the second-order rate constant k_{cat}/k_M for the reaction of the nonphysiological substrate LGAP compared to DGAP.

Estimated kinetic parameters for 3-quinuclidinone catalysis of LGAP isomerization are $K_d = 230$ M and $k_{cat} = 0.4\ s^{-1}$ (Richard, 1984). Comparison with the enzymatic values shows that k_{cat} for the enzymatic reaction is 100 times slower than quinuclidinone deprotonation of substrate within a weak encounter complex, suggesting that catalysis of the enzymatic reaction is due to slow, adventitious substrate deprotonation by a base at the catalytic site. Discussion of the stereochemistry of the enediol(ate) intermediate for enzymatic catalysis of LGAP reaction awaits the determination of the stereochemistry of proton incorporation into C-3 of DHAP.

ACKNOWLEDGMENTS

I thank Dr. Irwin Rose for his support.

Registry No. LGAP, 20283-52-7; triosephosphate isomerase, 9023-78-3; triose 1,2-enediol 3-phosphate, 57-04-5.

REFERENCES

Alber, T., Banner, D. W., Bloomer, A. C., Petsko, G. A., Phillips, D., Rivers, P. S., & Wilson, I. A. (1981) *Philos. Trans. R. Soc. London, Ser. B 293*, 159.

Bonsignore, A., Leoncini, G., Siri, A., & Ricci, D. (1973) *Ital. J. Biochem. 22*, 131.

Chiang, Y., Kresge, A. J., Tang, Y. S., & Wirz, J. (1984) *J. Am. Chem. Soc. 106*, 460.

Davies, C. S. (1962) *Ion Association*, pp 77–87, Butterworth, London.

Hall, A., & Knowles, J. R. (1975) *Biochemistry 14*, 4348.

Hammes, G. G., & Schimmel, P. R. (1970) *Enzymes, 3rd Ed. 2*, 67.

Hine, J. (1975) *Structural Effects on Equilibria in Organic Chemistry*, p 120, Wiley, New York.

Iyengar, R., & Rose, I. A. (1981) *Biochemistry 20*, 1229.

Iyengar, R., & Rose, I. A. (1983) *J. Am. Chem. Soc. 105*, 3301.

Knowles, J. R., & Albery, W. J. (1977) *Acc. Chem. Res. 10*, 105.

Kuo, D. J., O'Connell, E. L., & Rose, I. A. (1979) *J. Am. Chem. Soc. 101*, 5025.

McVittie, J. D., Esnouf, M. P., & Peacocke, A. R. (1972) *Eur. J. Biochem. 29*, 67.

Reynolds, S. J., Yates, D. W., & Pogson, C. I. (1971) *Biochem. J. 122*, 285.

Richard, J. P. (1984) *J. Am. Chem. Soc. 106*, 4926.

Rieder, S. V., & Rose, I. A. (1959) *J. Biol. Chem. 234*, 1007.

Rose, I. A. (1962) *Brookhaven Symp. Biol. 15*, 293.

Rose, I. A. (1982) *Methods Enzymol. 87*, 84–97.

Rose, I. A. (1984a) *J. Am. Chem. Soc. 106*, 6117.

Rose, I. A. (1984b) *Biochemistry 23*, 5893.

Rose, I. A., & O'Connell, E. L. (1969) *J. Biol. Chem. 244*, 6548.

Schray, K. J., O'Connell, E. L., & Rose, I. A. (1973) *J. Biol. Chem. 248*, 2214.

Trentham, D. R., Murray, C. H., & Pogson, C. I. (1969) *Biochem. J. 114*, 19.

[4] There is a small KCl salt effect that leads to a 40% increase in the rate constants for LGAP elimination and isomerization as the KCl concentration is decreased from 0.9 to 0 M. The ratio for enediolate partitioning between elimination and isomerization product formation calculated from the ratio of these rate constants is therefore independent of KCl concentration.

[5] The K_d value can be used to calculate that 10 mM LGAP will inhibit by 25% triosephosphate isomerase catalysis of the reaction of 1 mM DGAP [K_m for DGAP is 0.3 mM (Reynolds et al., 1971)], whereas only 10% inhibition was observed (see Results). The difference can reasonably be attributed to experimental error in the K_d determination from a two-point double-reciprocal plot.