Kinetic Parameters for the Elimination Reaction Catalyzed by Triosephosphate Isomerase and an Estimation of the Reaction's Physiological Significance†,‡

John P. Richard
Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506-0055
Received October 25, 1990; Revised Manuscript Received January 22, 1991

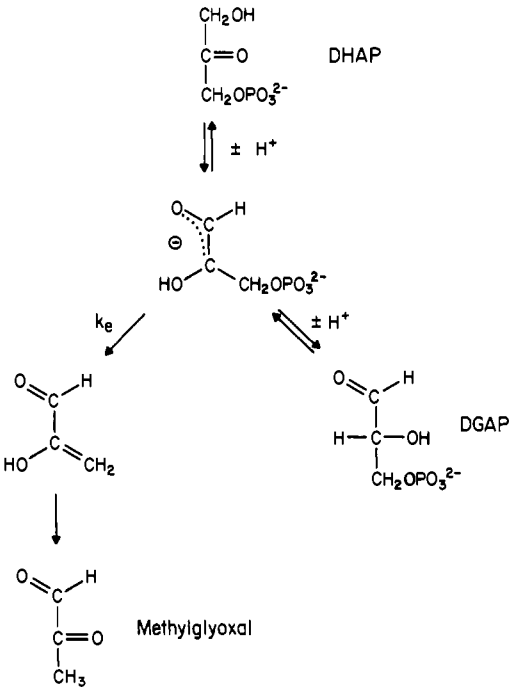
ABSTRACT: Kinetic parameters for triosephosphate isomerase catalysis of the elimination reaction of an equilibrium mixture of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (DGAP) to form methylglyoxal and phosphate ion are reported for the enzyme from rabbit muscle. Pseudo-first-order rate constants for the disappearance of substrate (k_{elim}) were determined for reactions at [Enzyme] ≫ [Substrate]. The second-order rate constant k_{Enz} = 10.1 M^{-1} s^{-1} was determined from a plot of k_{elim} against enzyme concentration. The kinetic parameters, determined from a steady-state kinetic analysis at [Substrate] ≫ [Enzyme], are k_{cat} = 0.011 s^{-1}, K_m = 0.76 mM, and k_{cat}/K_m = 14 M^{-1} s^{-1}. The estimated rate-constant ratio for partitioning of the enzyme-bound intermediate between protonation at carbon 2 and elimination, 1 000 000, is much larger than the ratio of 6.5 determined for the reaction of the enediolate phosphate in a loose complex with quinuclidinonium cation, a small buffer catalyst. There is a 10^5–10^8-fold decrease in the rate constant for the elimination reaction of the enediolate phosphate when this species binds to triosephosphate isomerase. The kinetic parameters for the elimination reaction catalyzed by the native triosephosphate isomerase and for the reaction catalyzed by a mutant form of the enzyme, which is missing a segment that forms hydrogen bonds with the phosphate group of substrate [Pompliano, D. L., Peyman, A., & Knowles, J. R. (1990) *Biochemistry 29*, 3186–3194] are similar. This is attributed to offsetting effects of the deletion, which decrease the steady-state concentration of the enzyme-bound intermediate and increase the rate constant for its breakdown to form elimination products. The kinetic parameters were used to calculate a cellular velocity of 0.4 mM methylglyoxal/day for the formation of methylglyoxal from the elimination reaction catalyzed by triosephosphate isomerase. This calculation suggests that an important function for glyoxalases I and II is to metabolize the methylglyoxal that is produced by triosephosphate isomerase.

Triosephosphate isomerase has, by several criteria, achieved perfection as a catalyst of the interconversion of substrates dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde-3-phosphate (DGAP) (Knowles & Albery, 1977). Therefore, it is surprising that the enzyme also catalyzes the degradation of these substrates to give methylglyoxal and phosphate (Browne et al., 1976; Webb et al., 1977; Iyengar & Rose, 1981a).

Studies on the nonenzymatic reactions of triosphosphates have shown that triosephosphate isomerase is a victim of its enediol(ate) phosphate intermediate, which is highly labile to the elimination of the phosphate ion (k_e, Scheme I).[1] In water, the enediolate phosphate dianion and monoanion react with estimated rate constants k_e of 8 × 10^6 s^{-1} and 8 × 10^8 s^{-1}, respectively (Richard, 1984). These species are in rapid equilibrium with the enediol, and this, in turn, has a half-life of ~1 μs in water at pH 7 (Richard, 1985). Triosephosphate isomerase, with the aid of a flexible loop that surrounds the phosphate group of the enzyme-bound intermediate (Banner, et al., 1975; Alber et al., 1981; Pompliano et al., 1990), does a less than perfect job of suppressing this elimination reaction.

The elimination reaction catalyzed by triosephosphate isomerase is slow relative to the isomerization reaction and was detected only by chance in experiments where the substrate was incubated with large concentrations of the enzyme (Webb et al., 1977). Despite the slow rate of the reaction, its mechanistic and physiological consequences are probably quite significant.

Scheme I



(1) The rapid rate of enediolate phosphate decomposition in water raises several questions about the details of the mechanism for the suppression of the elimination reaction of

† This work was supported by National Institutes of Health Grants GM 39754, GM 20940, CA-06927, and RR-005539 and by an appropriation from the Commonwealth of Pennsylvania.

‡ First presented at the 14th International Congress of Biochemistry, Prague, Czechoslovakia (Richard, 1988).

[1] It is not known whether this intermediate is bound to triosephosphate isomerase as the enediol or the enediolate (Petsko et al., 1984; Richard, 1987).

0006-2960/91/0430-4581$02.50/0 © 1991 American Chemical Society

the enzyme-bound enediol(ate) intermediate. How large is the change in reactivity of the enediol(ate) upon binding to the isomerase? By what mechanism does the enzyme stabilize the bound enediol(ate) toward breakdown by β-elimination of phosphate? How do the kinetic parameters for the elimination reaction change upon deletion, by site-directed mutagenesis, of a flexible loop that is essential for effective catalysis of the isomerization reaction? This loop forms strong interactions with the phosphate group of the enzyme-bound ligand, and these interactions inhibit the elimination of phosphate ion from the intermediate (Pompliano et al., 1990).

(2) Triosephosphate isomerase is an extremely effective catalyst of the isomerization reaction, and the enzyme is present at very high cellular concentrations (Shonk & Boxer, 1964). Therefore, an enzyme-catalyzed elimination reaction that is very slow relative to the fast isomerization reaction still might generate significant concentrations of cellular methylglyoxal. The kinetic instability of the enediolate phosphate, which at the very least constitutes a serious flaw in the "logic" of glycolysis (Walsh, 1979), may have far-reaching metabolic consequences. Triosephosphate isomerase catalyzes the formation of the enediol(ate) phosphate, and this intermediate, unavoidably, breaks down to an enolaldehyde that then tautomerizes to methylglyoxal (Scheme I). Methylglyoxal is a highly reactive compound, with no known biological function, that is probably toxic (Carrington & Douglas, 1986). This compound is metabolized to D-lactate by the action of glyoxalases I and II (Racker, 1955). Many metabolic roles have been considered for the glyoxalase enzymes, but their true function remains an enigma (Carrington & Douglas, 1986). One function for these enzymes, which has not been previously been considered, may be to remove methylglyoxal that forms as a byproduct of an enzyme-catalyzed reaction.

I report here the kinetic parameters for the elimination reaction catalyzed by triosephosphate isomerase. These parameters are compared with data for the isomerization reaction catalyzed by the native enzyme, the isomerization reaction catalyzed by the small buffer quinuclidinone (Richard, 1984), and the isomerization and elimination reactions catalyzed by a mutant form of triosephosphate isomerase that is much less effective than the native enzyme at supressing the elimination reaction of the bound intermediate (Pompliano et al., 1990). The results provide support for the proposal that the triosephosphate isomerase catalyzed elimination reaction is an important source of cellular methylglyoxal.

Materials and Methods

Many of the materials and methods used in this work have been described earlier (Richard, 1984, 1985). Rabbit muscle triosephosphate isomerase (9000 units/mg) was purchased from Calbiochem-Behring. The enzyme was assayed by coupling the isomerization of DGAP to the oxidation of NADH by using α-glycerolphosphate dehydrogenase. The concentration of enzyme subunits was calculated from the absorbance at 280 nm and an extinction coefficient of 3.1×10^4 M^{-1} cm^{-1} that was calculated for a subunit molecular weight of 26 000 Da (McVittie et al., 1972). Reaction mixtures were prepared with 0.10 M triethanolamine·HCl. Triosephosphate isomerase, which was purchased as an ammonium sulfate precipitate, was dialyzed against a stock solution of this buffer. In some experiments it was necessary to correct the concentration of triosephosphate isomerase ($\leq$40%) for the loss in specific activity that occurred on standing for up to 3 days at 0 °C. DHAP was prepared by acid-catalyzed hydrolysis of the ketal and was stored in dilute acid at −15 °C (Richard, 1984). The pH of the solution of DHAP was adjusted to 7.9 immediately before use.

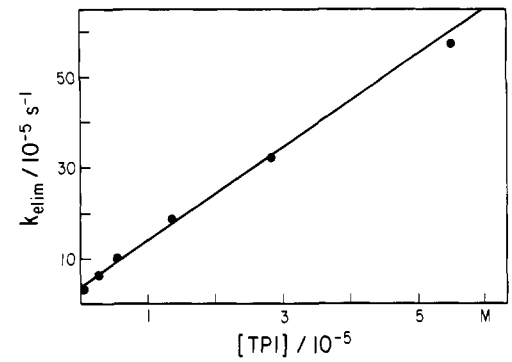


FIGURE 1: The increase in the rate constant k_{elim} with increasing concentrations of triosephosphate isomerase for an experiment in which the concentration of the enzyme is kept in great excess over the initial substrate concentration of ca. 10 nM. The data point that lies closest to the y axis is for a reaction in the presence of 3×10^{-7} M triosephosphate isomerase.

Elimination Reaction of an Equilibrium Mixture of DHAP and DGAP. The kinetic parameters for this reaction were determined by two different methods.

(*A*) *Enzyme in Excess over Substrate.* Reaction mixtures (0.50 mL) that contained triethanolamine·HCl (0.10 M, pH 7.9) and triosephosphate isomerase [$(0.03–5) \times 10^{-5}$ M] were prepared and incubated at 39 °C for 10 min. The reactions were then initiated by the addition of 5 μL of [^{32}P]DHAP ($\approx$5 pmol, 5×10^6 cpm). At recorded times, 5-μL aliquots were withdrawn, and the organic and inorganic phosphates were separated by extraction of the molybdate complex of inorganic phosphate into isobutanol (Iyengar & Rose, 1981b). Pseudo-first-order rate constants, k_{elim}, were determined from the slopes of plots of six or more values of ln $(C_t - C_\infty)$ against time, where C_t are the counts remaining in the aqueous layer at a given time and C_∞ are the counts remaining in the aqueous layer at t_∞. Semilogarithmic plots of the data were linear for 2–3 half-times for the reaction.

(*B*) *Substrate in Excess over Enzyme.* The components of this reaction were incubated at 39 °C for 10 min before mixing. Triethanolamine·HCl (0.38 mL, 0.10 M at pH 7.9) and 0.02 mL of triosephosphate isomerase (650 units for DGAP isomerization, final concentration = 5.5×10^{-6} M) were mixed, and dihydroxyacetone phosophate (0.018 M, pH 7.9) and water were added in varying proportions to give solutions with final volumes of 0.5 mL ([triethanolamine·HCl] = 0.08 M). The reactions were initiated by the addition of 5 μL of [^{32}P]DHAP (5 pmol, $\approx 5 \times 10^6$ cpm). At recorded times, 5-μL aliquots were withdrawn, and inorganic phosphate was separated from the unreacted substrate (Iyengar & Rose, 1981b). The initial velocity (v_0) of the reaction was determined from the slope of a plot of the concentration of inorganic phosphate against time (5 points during the first 10% of reaction). The velocity of the enzyme-catalyzed elimination reaction (v_E) was calculated as $v_0 - v_N$, where v_N is the velocity of the nonenzymatic reaction. The value for v_N was calculated as the product of $k_N = 3.0 \times 10^{-5}$ s^{-1} for the reaction at 3×10^{-7} M triosephosphate isomerase (data point closest to the y axis, Figure 1)[2] multiplied by the concentration of substrate.

[2] This value for k_N includes a small (10%) correction. This was required because k_N estimated by extrapolation was for a reaction in 0.10 M triethanolamine (see Figure 1), instead of 0.08 M buffer. Triethanolamine is a general base catalyst of the elimination reaction of DHAP, and a decrease from 0.10 to 0.08 M triethanolamine at pH 7.9 will cause a 10% decrease in the pseudo-first-order rate constant for the elimination reaction (Richard, 1984).

Table I: Kinetic Parameters for Catalysis of the Reactions of Dihydroxyacetone Phosphate

catalyst	reaction	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m (M^{-1} s^{-1})	$(k_{C_2}/k_e)^a$
native isomerase	DHAP → DGAP[b]	900	6.2×10^{-4}	6×10^4	
	DHAP + DGAP → methylglyoxal	0.011[c] (0.006)[d]	7.6×10^{-4}	12 ± 2[g]	$\sim 10^6$[h]
mutant isomerase[e]	DHAP → DGAP	0.0081	8.2×10^{-3}	1.0	
	DHAP → methylglyoxal	0.0053	8.2×10^{-3}	0.64	1.5[i]
quinuclidinone[f]	DHAP → DGAP	0.0015	21	7×10^{-5}	6.5[j]

[a] Ratio of the rate constants for the partitioning of the reaction intermediate between protonation to form DGAP (k_{C_2}) and elimination to form methylglyoxal (k_e). [b] Data from Krietsch et al. (1970). [c] Observed value of k_{cat} for enzymatic catalysis of the reaction of an equilibrium mixture of DHAP and DGAP. [d] Calculated value for enzymatic catalysis of the reaction of DHAP (see text). [e] Data from Pompliano et al. (1990). [f] Data from Richard (1984). [g] The average of values determined in experiments at [E] ≫ [S] and [S] ≫ [E]. [h] This ratio is 10 times larger than the ratio of the values of k_{cat} for the isomerization and elimination reactions because product release limits the overall rate of the isomerization reaction (see text). [i] The ratio of the values of k_{cat} for the isomerization and elimination reactions. [j] Calculated from $k_e = 8 \times 10^6$ s^{-1} and $k_{C_2} = 5.2 \times 10^7$ s^{-1} for the nonenzymatic reactions (Richard, 1984).

Results

The decomposition of DHAP and DGAP, at chemical equilibrium, to give inorganic phosphate and methylglyoxal follows first-order kinetics when the concentration of enzyme is kept at >30-fold excess over substrate. Figure 1 shows the increase in the observed pseudo-first-order rate constants, k_{elim}, at increasing concentrations of triosphosphate isomerase subunits. The slope of the line that correlates the data at [E] ≤ 2.8×10^{-5} M is $k_{Enz} = 10.1$ M^{-1} s^{-1}, the second-order rate constant for the enzyme-catalyzed reaction. The small negative deviation of the value for k_{elim} at 5.5×10^{-5} M enzyme subunits is consistent with the onset of saturation of the substrate by the enzyme.

Enzymatic catalysis of the elimination reaction was also studied under the conventional conditions, where the concentration of the substrate is kept in excess over that of the enzyme. Triosephosphate isomerase (5.5×10^{-6} M) was present at a sufficiently large concentration to establish equilibrium for the isomerization reaction, before significant formation of the elimination products occurs; at [E] = 2×10^{-7} M, equilibrium for the isomerization reaction is established within 10 min (Veech et al., 1969). The initial velocity of the enzyme-catalyzed elimination reaction, v_E, increases with increasing concentrations of substrate to a maximal velocity (data not shown). The velocity, v_E, is calculated as the difference between the observed velocity, v_0, and that for the nonenzymatic reaction, v_N. At the lowest concentration of substrate (0.072 mM), v_E is 72% of v_0; the contribution of v_E decreases to 44% of v_0 when the concentration of substrate is increased to 1.8 mM. Figure 2 shows the Lineweaver–Burk plot of the data; the slope and the intercept of this plot give the following kinetic parameters for the enzyme-catalyzed elimination reaction: $k_{cat} = 0.011$ s^{-1}, $K_m = 0.76$ mM,, and $k_{cat}/K_m = 14$ M^{-1} s^{-1}.

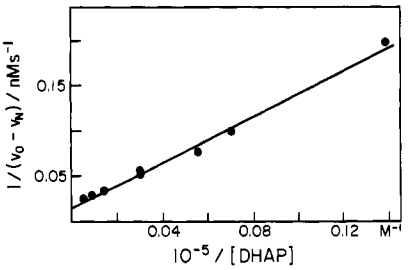


FIGURE 2: A Lineweaver–Burk plot of initial velocity data for the enzyme-catalyzed elimination reaction ([DHAP] ≫ [E]). The substrate is added to the reaction mixture as DHAP and is rapidly converted by triosephosphate isomerase to an equilibrium mixture of DHAP and DGAP.

Discussion

When the concentration of triosephosphate isomerase is kept in great excess over the concentration of substrate, the breakdown of substrate to elimination products follows pseudo-first-order kinetics. The second-order rate constant for the reaction, k_{Enz}, was calculated from the slope of a plot of k_{elim} (s^{-1}) against enzyme concentration ($k_{Enz} = 10.1$ M^{-1} s^{-1}, Figure 1). This second-order rate constant was also determined from the increase, with increasing substrate concentration, in the initial velocity for the reaction at [S] ≫ [E] ($k_{cat}/K_m = 14$ M^{-1} s^{-1}). There is acceptable agreement between the values for the rate constant determined in these experiments.

These reactions were initiated by the addition of DHAP, which was brought to equilibrium with its isomer DGAP before significant elimination occurred. Hence, the reactions of both DHAP and DGAP contribute to observed rate constant of $(k_{cat})_{obsd} = 0.011$ s^{-1}. The observed rate constant is equal to $(k_{cat})_{DHAP} f(\text{E·DHAP}) + (k_{cat})_{DGAP} f(\text{E·DGAP})$, where f(E·DHAP) and f(E·DGAP) are the fractions of the total enzyme that forms complexes with DHAP and DGAP, respectively. The concentration of DHAP at chemical equilibrium is much greater than that of DGAP, but DGAP is the more reactive substrate in the isomerization reaction, so that the reactions of DHAP and DGAP from solution are expected to make roughly equal contributions to $(k_{cat})_{obsd}$. A value of $(k_{cat})_{DHAP} = 0.006$ s^{-1} for the reaction of DHAP was estimated from $(k_{cat})_{obsd}$, the ratio of the concentrations of triosephosphates at chemical equilibrium ($[DHAP]_{eq}/[DGAP]_{eq}$ = 22) (Veech et al., 1969), and with the following assumptions: (1) the relative values of K_m and k_{cat} for DHAP and DGAP in the elimination reaction are the same as those measured for the isomerization reaction, $(K_m)_{DGAP}/(K_m)_{DHAP} = 0.5$ and $(k_{cat})_{DGAP}/(k_{cat})_{DHAP} = 10$ (Krietsch et al., 1970), and (2) the ratio of the K_m values for the elimination reaction of DGAP and DHAP is equal to the ratio of the dissociation constants for the two substrates.

The kinetic parameters for the elimination and isomerization reactions of DHAP catalyzed by wild-type triosephosphate isomerase from rabbit muscle and by a deletion mutation of the wild-type enzyme from chicken muscle (Pompliano et al., 1990) are given in Table I. This table also gives the kinetic parameters for the isomerization reaction catalyzed by quinuclidinone (Richard, 1984) and the estimates for the rate-constant ratios k_{C_2}/k_e for protonation of the enediol(ate) intermediate at carbon 2 (k_{C_2}) and the elimination of phosphate ion (k_e) for the reactions of the enediolate complexed to the respective enzymes and in a loose association complex with the quinuclidinonium cation.

The ratio k_{C_2}/k_e for the mutant enzyme was calculated from the ratio of the values of k_{cat} for the enzyme-catalyzed isomerization and elimination reactions, with the assumption that the products of these two reactions form by partitioning of a

common enediol(ate) intermediate.

The value of k_{cat} for the isomerization reaction catalyzed by the native enzyme is limited by the rate of release of product DGAP to solution, while the value for k_e is almost certainly limited by the rate of expulsion of the phosphate ion from the enediolate. The rate constant for protonation of the enediol(ate) at carbon 2 is ca. 10-fold *greater than* that for the release of DGAP (Knowles & Albery, 1977). Therefore, k_{C2}/k_e for the native enzyme is estimated to be 10-fold larger than the ratio of values for k_{cat} for the isomerization and elimination reactions (Table I).

The rate constant ratio of k_{C2}/k_e of $\sim 10^6$ for the native enzyme and an upper limit of $k_{C2} \leq 5 \times 10^7$ s^{-1} for protonation of the enzyme-bound intermediate[3] gives $k_e \leq 5 \times 10^1$ s^{-1}. The lower limit of $k_{C2} \geq 6 \times 10^4$ s^{-1} (Knowles & Albery, 1977) gives $k_e \geq 0.06$ s^{-1}. These values are much smaller than $k_e \approx 8 \times 10^6$ s^{-1} estimated for the nonenzymatic reaction in water (Richard, 1984), and they establish a range of 10^5–10^8-fold for the stabilization of the enediolate phosphate toward elimination upon binding to triosephosphate isomerase.

The kinetic parameters for triosephosphate isomerase from rabbit muscle and chicken muscle are similar (Pompliano et al., 1990; Krietsch et al., 1970), and there is no evidence for significant differences in the mechanism of the enzymes from the two sources. The mutant form of the enzyme from chicken muscle (Table I) is lacking part of a loop that forms strong interactions with the phosphate group of enzyme-bound intermediate. The comparison of kinetic parameters for the native and mutant enzymes shows the contribution of these interactions to the suppression of the elimination reaction of enzyme-bound intermediate and to the overall catalysis of the isomerization reaction.

The values of k_{cat} for the elimination reactions catalyzed by the native and mutant forms of triosephosphate isomerase are nearly the same (Table I). The rate constant k_{cat} for the elimination reaction is equal to $k_e f_X$, where k_e is the microscopic rate constant for the breakdown of the enzyme–intermediate complex to form elimination products, and f_X is the fraction of the enzyme present as this complex at a saturating concentration of substrate. There is good evidence that the deletion mutation causes a decrease in the stability of the enzyme-bound intermediate relative to the substrate, so that f_X is smaller than that for the native enzyme (Pompliano et al., 1990). Apparently, the decrease in f_X is offset by an increase in k_e, so that the mutation has little effect on the observed catalytic rate constant for the elimination reaction.

The reactivity of DHAP in a complex with the mutant enzyme, as measured by the turnover number k_{cat}, is similar to the reactivity of DHAP in a complex with quinuclidinone; and the reactivity of the enediol(ate) phosphate at the mutant enzyme, as measured by k_{C2}/k_e, is similar to its reactivity in a complex with quinuclidinonium cation (Table I). These data suggest that the interactions of the essential loop in the native enzyme with the phosphate group are largely responsible for the increase in the reactivity of the substrates for deprotonation at the carbon and the decrease in the reactivity of the enediolate for expulsion of phosphate ion that occur on the binding of these ligands to the enzyme.

The phosphate group of the bound intermediate is immobilized in the binding pocket by interactions with the essential loop. Suppression of the elimination reaction would result if unfavorable steric interactions were to develop between the enolaldehyde and the phosphate ion upon cleavage of the C–OP$_i$ bond (Pompliano et al., 1990), if the phosphate group were "locked" into a position that minimizes delocalization of electrons from the π-orbital of the enolate to the orbital that develops on cleavage of the C–OP$_i$ bond (Alber et al., 1981) or if the O-protonated enediol were the predominate form of the enzyme-bound intermediate.

There are at least two ways that binding interactions between the enzyme and the phosphate group of substrates might be "utilized" (Jencks, 1975) to stabilize the transition state for the deprotonation of triosephosphates at carbon atoms several angstroms removed from the site of the binding interactions.

(1) There is evidence that the essential loop forms stronger interactions with the enediolate phosphate analogue phosphoglycolate than with the substrate (Pompliano et al., 1990). Differential binding interactions that stabilize the intermediate more than the substrate will contribute to catalysis to the extent that the preferential binding of the endiol(ate) phosphate is expressed at the transition for its formation from substrate.

(2) The binding interactions may hold the carbonyl group of the substrate in a position to hydrogen bond with His-95 and Lys-13 (Alber et al., 1981; Petsko et al., 1984; Nickbarg et al., 1988). This could contribute to catalysis of deprotonation of the carbon by polarization of the carbonyl group, which would bring the charge at the carbonyl oxygen closer to the value of −1 for the enolate oxyanion (Alagona et al., 1984; Belasco & Knowles, 1980; Webb & Knowles, 1974), and by provision of an environment for the enediolate oxyanion that is similar to the polar environment in water, but without the unfavorable requirement for the loss in the translational and rotational entropy of up to three water molecules (Jencks, 1975).

Physiological Significance. The kinetic parameters for the elimination reaction determined in this work and the values for the cellular concentrations of triosephosphates (Willamson, 1965; Knowles & Albery, 1977) and triosephosphate isomerase (Shonk & Boxer, 1964) were used to calculate a velocity of 0.4 mM/day for methylglyoxal formation from the elimination

$$v_E = (k_{cat}/K_m)[\text{DHAP} + \text{DGAP}][\text{TPI}]$$
$$= (12\ \text{M}^{-1}\ \text{s}^{-1})(4 \times 10^{-5}\ \text{M})(1 \times 10^{-5}\ \text{M})(86{,}400\ \text{s/day})$$
$$= 0.4\ \text{mM/day} \quad (1)$$

$$v_N = (k_N)([\text{DHAP} + \text{DGAP}])$$
$$= (3.0 \times 10^{-5}\ \text{s}^{-1})(4 \times 10^{-5}\ \text{M})(86{,}400\ \text{s/day}) =$$
$$0.1\ \text{mM/day} \quad (2)$$

reaction catalyzed by triosephosphate isomerase (eq 1).[4]

[3] The estimated first-order rate constant for proton transfer from the quinuclidinonium cation (the conjugate acid of the tertiary amine quinuclidinone) to the enediolate phosphate dianion within an association complex of the two molecules (Richard, 1984). This is an upper limit. The enediolate is stabilized relative to the substrates upon binding to triosephosphate isomerase (Wolfenden, 1969, 1970; Collins, 1974; Pompliano et al., 1990), and this should lead to a decrease in the rate constant for the protonation of the enzyme-bound intermediate.

[4] The following are the values used for eq 1; $k_{cat}/K_m = 12 \pm 2$ M^{-1} s^{-1} is the second-order rate constant for the enzyme-catalyzed elimination reaction, [DGAP + DHAP] = 4×10^{-5} M is the cellular concentration of triosephosphates (Williamson, 1965; Knowles & Albery, 1977), and [TPI] = 1×10^{-5} M is the concentration of triosephosphate isomerase in rat skeletal muscle tissue. An activity of 2650 IU/g wet tissue has been reported (Shonk & Boxer, 1964). However, these activities were determined at 0.62 mM DGAP, while the specific activity (IU/mg protein) of triosephosphate isomerase was determined for a reaction at a saturating concentration of substrate. An activity of 4000 IU/g wet tissue would have been observed for an assay at [DGAP] $\gg K_m = 0.32$ mM (Krietsch et al., 1970). The tissue concentration of TPI was calculated by using a specific activity of 9000 IU/mg for the homogeneous enzyme and a molecular weight of 50 000 Da (Krietsch et al., 1970) and by assuming a density of 1.1 g/mL for rat muscle tissue.

Methylglyoxal is a compound with no known metabolic function that is thought to be toxic to the organism (Carrington & Douglas, 1986). If it were left to accumulate, the concentration of methylgloxal produced by triosephosphate isomerase would approach that of cellular triosephosphates in about 2 h. Fortunately, methylglyoxal is metabolized to D-lactate by glyoxalases I and II, a pair of enzymes also with no known metabolic function (Carrington & Douglas, 1986). It may be that the glyoxalase pathway has evolved for the purpose of removing methylglyoxal, which is formed as a byproduct of the triosephosphate isomerase reaction. If so, then it is not surprising that the role of these enzymes in metabolism has remained an enigma for so many years (Carrington & Douglas, 1986). The disposal of toxic byproducts is often required in the industrial production of chemicals, but it is unusual to encounter this problem along a metabolic pathway.

The formation of methylglyoxal from DGAP and DHAP has been studied in dialyzed whole cell homogenates of rat liver (Sato et al., 1980). The ratio of the velocities for methylglyoxal formation observed in the native dialyzate and in a dialyzate pretreated at 60 °C for 30 min are 2.8 and 8.8, respectively, at close to saturating concentrations for DGAP and DHAP as substrates. This is an estimate of the relative velocities for the enzyme-catalyzed and nonenzymatic reactions. These values are similar to the ratio $v_E/v_N = 4.0$ calculated from eq 1 and 2.[5] It is impossible to draw firm conclusions from comparisons of experiments performed in different laboratories, under different conditions, on enzymes from different tissues. However, these results are at least consistent with the proposal that the triosephosphate-isomerase-catalyzed elimination reaction is a major source of cellular methylglyoxal.

Experiments are planned to isolate from rat liver homogenates the protein or proteins that catalyze the elimination reaction of triosephosphates (Sato et al., 1980), in order to determine if this protein is triosephosphate isomerase and/or if there is a separate methylglyoxal synthase activity in these tissues (Hopper & Cooper, 1972; Cooper, 1974; Tsai & Gracy, 1976).

Acknowledgments

I thank Dr. Irwin Rose for his support during the time that these experiments were performed in his laboratory.

Registry No. DHAP, 57-04-5; DGAP, 591-57-1; triosephosphate isomerase, 9023-78-3; methylglyoxal, 78-98-8; glyoxalase I, 9033-12-9; glyoxalase II, 9025-90-5.

References

Alagona, G., Desmeules, P., Ghio, C., & Kollman, P. (1984) *J. Am. Chem. Soc. 106*, 3523–3632.

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 B 293*, 159–171.

Banner, D. W., Bloomer, A. C., Petsko, G. A., Phillips, D. C., Pogson, C. I., & Wilson, I. A. (1975) *Nature (London) 255*, 609–614.

Belasco, J. G., & Knowles, J. R. (1980) *Biochemistry 19*, 472–477.

Browne, C. A., Campbell, I. D., Kiener, P. A., Phillips, D. C., Waley, S. G., & Wilson, I. A. (1976) *J. Mol. Biol. 100*, 319–343.

Carrington, S. J., & Douglas, K. T. (1986) *IRCS Med. Sci. 14*, 763–768.

Collins, K. D. (1974) *J. Biol. Chem. 249*, 136–142.

Cooper, R. A. (1974) *Eur. J. Biochem. 44*, 81–86.

Hopper, D. J., & Cooper, R. A. (1972) *Biochem. J. 128*, 321–329.

Iyengar, R., & Rose, I. A. (1981a) *Biochemistry 20*, 1223–1229.

Iyengar, R., & Rose, I. A. (1981b) *Biochemistry 20*, 1229–1235.

Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol. 43*, 219–410.

Knowles, J. R., & Albery, W. J. (1977) *Acc. Chem. Res. 10*, 105–111.

Krietsch, W. K. G., Pentchev, P. G., Klingenburg, H., Hofstätter, T., & Bucher, T. (1970) *Eur. J. Biochem. 14*, 289–300.

McVittie, J. D., Esnouf, M. P., & Peacocke, A. R. (1972) *Eur. J. Biochem. 29*, 67–73.

Nickbarg, E. B., Davenport, R. C., Petsko, G. A., & Knowles, J. R. (1988) *Biochemistry 27*, 5948–5960.

Petsko, G. A., Davenport, R. C., Fraenkel, D., & Rajbhandary, U. L. (1984) *Biochem. Soc. Trans. 12*, 229–232.

Pompliano, D. L., Peyman, A., & Knowles, J. R. (1990) *Biochemistry 29*, 3186–3194.

Racker, E. (1955) *Glutathione, A Symposium*, p 407, Academic Press, New York.

Richard, J. P. (1984) *J. Am. Chem. Soc. 106*, 4926–4936.

Richard, J. P. (1985) *Biochemistry 24*, 949–953.

Richard, J. P. (1987) in *Enzyme Mechanisms* (Page, M. I., & Williams, A., Eds.) pp 298–316, The Royal Chemical Society, London.

Richard, J. P. (1988) Abstract Th:002 presented at the 14th International Congress of Biochemistry, July 10–15, Prague, Czechoslovakia.

Sato, J., Wang, Y.-M., & van Eys, J. (1980) *J. Biol. Chem. 255*, 2046–2050.

Shonk, C. E., & Boxer, G. E. (1964) *Cancer Res. 24*, 709–721.

Tsai, P.-K., & Gracy, R. W. (1976) *J. Biol. Chem. 251*, 364–367.

Veech, R. L., Raijman, L., Dalziel, K., & Krebs, H. A. (1969) *Biochem. J. 115*, 837–842.

Walsh, C. (1979) *Enzymatic Reaction Mechanisms*, pp 891–929, W. H. Freeman, San Francisco.

Webb, M. R., & Knowles, J. R. (1974) *Biochem. J. 141*, 589–592.

Webb, M. R., Standring, D. N., & Knowles, J. R. (1977) *Biochemistry 16*, 2738–2741.

Williamson, J. R. (1965) *J. Biol. Chem. 240*, 2308–2321.

Wolfenden, R. (1969) *Nature (London) 223*,, 704–705.

Wolfenden, R. (1970) *Biochemistry 9*, 3404–3407.

[5] Calculated by using $k_N = 3.0 \times 10^{-5}$ s^{-1} (Figure 1) and [DGAP + DHAP] = 4×10^{-5} M (Williamson, 1965; Knowles & Albery, 1977).