Structure of the Triosephosphate Isomerase–Phosphoglycolohydroxamate Complex: An Analogue of the Intermediate on the Reaction Pathway†

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ABSTRACT: The glycolytic enzyme triosephosphate isomerase (TIM) catalyzes the interconversion of the three-carbon sugars dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) at a rate limited by the diffusion of substrate to the enzyme. We have solved the three-dimensional structure of TIM complexed with a reactive intermediate analogue, phosphoglycolohydroxamate (PGH), at 1.9-Å resolution and have refined the structure to an *R*-factor of 18%. Analysis of the refined structure reveals the geometry of the active-site residues and the interactions they make with the inhibitor and, by analogy, the substrates. The structure is consistent with an acid–base mechanism in which the carboxylate of Glu-165 abstracts a proton from carbon while His-95 donates a proton to oxygen to form an enediol (or enediolate) intermediate. The conformation of the bound substrate stereoelectronically favors proton transfer from substrate carbon to the syn orbital of Glu-165. The crystal structure suggests that His-95 is neutral rather than cationic in the ground state and therefore would have to function as an imidazole acid instead of the usual imidazolium. Lys-12 is oriented so as to polarize the substrate oxygens by hydrogen bonding and/or electrostatic interaction, providing stabilization for the charged transition state. Asn-10 may play a similar role.

Triosephosphate isomerase (TIM) catalyzes the central reaction in glycolysis, the interconversion of the triose phosphates dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) (Figure 1). Since only GAP can proceed further down the glycolytic pathway, the TIM reaction ensures efficient catabolism of all six carbons in glucose. The TIM reaction is at a branch point in sugar metabolism, from which biosynthetic as well as biodegradative pathways emanate. Consequently, there is strong evolutionary pressure for efficient catalysis by the enzyme under in vivo conditions. Viscosity-dependence studies have established that TIM is diffusion-controlled, that is, the rate of the reaction is limited by the diffusion of substrate and product to and from the active site; all of the purely chemical steps are faster (Blacklow et al., 1988).

As a possible example of an evolutionary perfect catalyst (Albery & Knowles, 1976a), TIM has been the subject of a variety of studies by physical and chemical means. TIM-catalyzed isomerization of DHAP and GAP occurs 10^9-fold faster than the nonenzymatic reaction catalyzed by carboxylate ion or other organic bases (Richard, 1984; Hall & Knowles, 1975). Isotope exchange studies suggested that a single enzymic base is required (Rieder & Rose, 1959); affinity labeling experiments (Coulson et al., 1970; Waley et al., 1970; Hartman, 1970) established that the base is the carboxylate side chain of Glu-165. Trapping experiments demonstrated the existence of an intermediate, either a *cis*-enediol phosphate or an enediolate phosphate, which could fragment to form methyl glyoxal and inorganic phosphate (Rose, 1982). TIM catalyzes both isomerization and fragmentation, but the latter is disfavored by almost 5 orders of magnitude. Elegant isotope labeling studies established the complete free energy profile for the TIM reaction (Albery & Knowles, 1976b). Spectroscopic studies suggested an electrophilic component on the enzyme that polarizes the carbonyl group of the bound substrate, thereby lowering the pK_a of the C1 hydrogen of DHAP (Belasco & Knowles, 1980).

The crystal structures of native chicken muscle and yeast TIM (Banner et al., 1975; Lolis et al., 1990), and the medium-resolution structure determination of the TIM–DHAP Michaelis complex (Alber et al., 1981), are in accord with these deductions from the solution studies and have allowed the active-site residues to be identified. In addition to Glu-165, the side chains of Lys-12 (Lys-13 in the chicken muscle enzyme sequence), Asn-10, His-95, Ser-96, and Glu-97 are all positioned so as to interact with the sugar portion of the substrate or with each other. Substrate binding induces a large conformational change in the enzyme: a loop of 10 amino acids, residues 168–177 in yeast TIM, moves by 8 Å as a rigid unit to fold down over the active site and interact with the phosphate of bound DHAP (Alber et al., 1981, 1987; Joseph et al., 1990).

These studies led to a more detailed mechanism for TIM catalysis that is in agreement with the earlier suggestions: Glu-165 acts as a general base to abstract the *pro-R* proton from C1 of DHAP, with electrophilic catalysis by His-95

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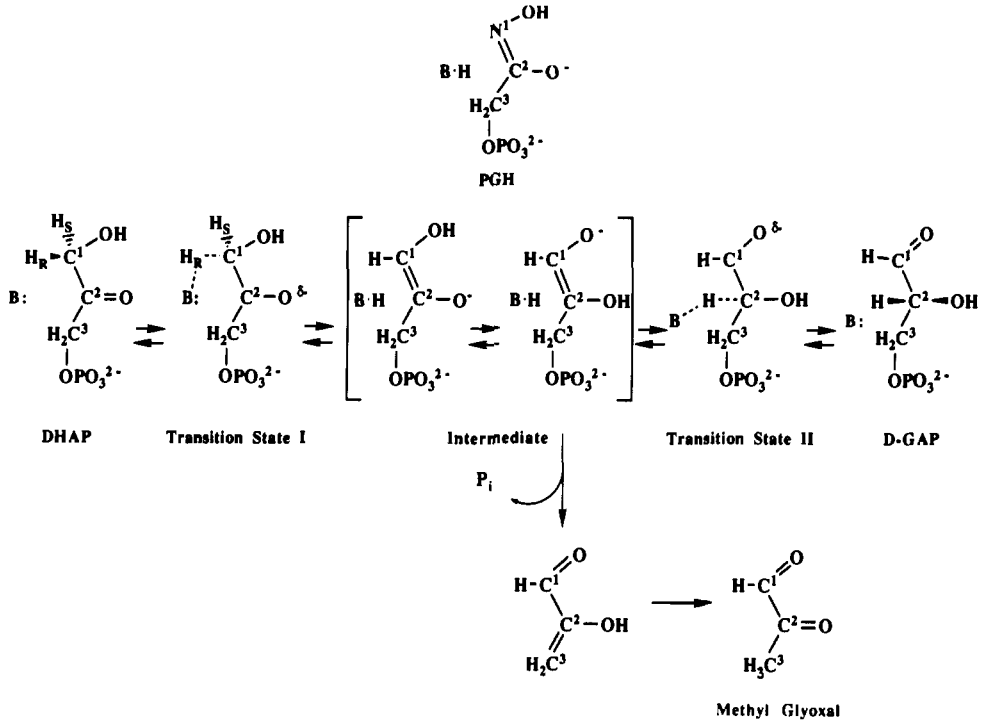

FIGURE 1: Reaction catalyzed by triosephosphate isomerase. Although the intermediate in the reaction is shown here as symmetrical *cis*-enediolate phosphates, it is possible that it is actually the protonated *cis*-enediol phosphate. Approximately 1 turnover in 100 000, the intermediate eliminates phosphate to form methyl glyoxal. The stereochemistry and charge configuration of phosphoglycolohydroxamate (PGH), which is also shown, is thought to resemble that of the putative endiolate intermediate or transition state of the isomerization reaction.

(Figure 2a) and Lys-12 serving to make the hydrogen more acidic and to stabilize the partial negative charge that forms on the carbonyl oxygen in the transition state. Formation of the intermediate might be aided by His-95 also acting as a general acid to donate a proton to substrate oxygen (Figure 2, panel b or c). Glu-165 then donates the proton to C2 to give GAP. The role of the flexible loop in this mechanism is to protect the active site from contact with bulk water and to prevent the elimination of phosphate from the intermediate (Alber et al., 1987; Pompliano et al., 1990).

Site-directed mutagenesis has been used to test these hypotheses. Replacement of Glu-165, or Lys-12, reduces or abolishes the catalytic activity of TIM (Strauss et al., 1985). Reduction of k_{cat} caused by the replacement of Glu-165 with Asp can be partially offset by the second-site substitution of Ser-96 with Pro (Blacklow & Knowles, 1990). Mutation of His-95 to Gln reduces k_{cat} in both directions by over 200-fold and also causes a change in the mechanism (Petsko et al., 1984; Nickbarg et al., 1988). Data from isotope exchange studies on this mutant are consistent with Glu-165 abstracting the *pro-R* proton from DHAP in the usual way but then delivering it to the carbonyl oxygen at C2 of the substrate to form the intermediate. Glu-165 then appears to deprotonate the hydroxyl group at C1 and transfer that proton to the C2 carbon to give GAP. This double role for Glu-165 in the H95Q mutant suggests that in the wild-type enzyme there is a requirement for acid catalysis by His-95 to form the intermediate. Presumably, the loss of His-95 in the mutant has forced the enzyme to find another acid to protonate the substrate carbonyl, and the carboxylate of Glu-165, which becomes a carboxylic acid after it picks up the *pro-R* hydrogen, is able to serve as a replacement. Thus, the data from the H95Q mutant are most consistent with the role for His-95 depicted in Figure 2, panel b or c (Komives et al., 1991).

A complete description of the TIM mechanism requires a detailed atomic model and associated energies for each species formed during the reaction. The intermediate is too short-lived for direct structural characterization, transition states are unobservable (at least crystallographically) by definition, and the crystals of the Michaelis complex prepared by diffusing DHAP into native yeast TIM crystals do not diffract to high enough resolution to allow the precise geometry of enzyme or substrate to be determined. We have recently determined the structure of yeast TIM cocrystallized with the putative transition-state analogue 2-phosphoglycolate at medium resolution (Lolis & Petsko, 1990), but this inhibitor lacks some of the structural features expected in the actual transition state or intermediate.

To see the active-site environment in a catalytically important conformation at high resolution, we have solved the structure of TIM cocrystallized with the tight-binding inhibitor phosphoglycolohydroxamate (PGH) (Collins, 1974). PGH is structurally analogous to an enediolate intermediate or enediolate-like transition state (Figure 1). The crystal structure of the TIM–PGH complex, reported here, is consistent with the general features of the accepted mechanism of the TIM reaction, but it reveals new details about the roles of specific residues. In particular, the structure suggests that the substrate and Glu-165 are oriented for efficient catalysis, and that His-95, if it is functioning as a general acid, is doing so in an unexpected form. The results presented in this paper have also provided the starting point for a combined quantum mechanics/molecular mechanics study of the TIM-catalyzed reaction, which is presented in the accompanying paper (Bash et al., 1991).

Experimental Procedures

Yeast TIM was crystallized in the presence of saturating amounts of PGH by precipitation with poly(ethylene glycol). Crystallization conditions are similar to those described for native yeast TIM (Alber et al., 1981) except for the presence of saturating amounts of PGH. Crystals of the TIM–PGH complex are not isomorphous with those of native yeast TIM. The TIM–PGH complex crystallizes in rectangular prisms

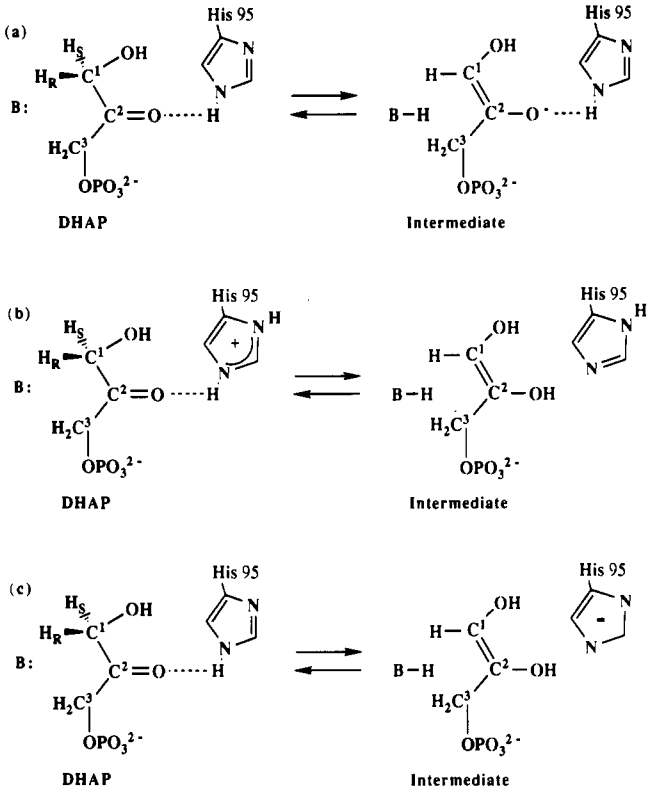

FIGURE 2: Possible roles of the side chain of His-95 in the catalytic mechanism of TIM. In (a) a neutral imidazole provides electrophilic stabilization of the transition state (or intermediate) by hydrogen bonding. In (b) a cationic imidazole functions as an acid to protonate the carbonyl group and produce an enediol. In (c) a neutral imidazole functions as an acid to protonate the carbonyl. The mechanistic data from the H950 mutant of yeast TIM would appear to favor (b) or (c), and consideration of the usual protonation states of histidine would lead one to expect that (b) would apply. The structure of the TIM–PGH complex, however, as shown in Figure 3, is most consistent with (c) or with a concerted version of (c).

having the symmetry of space group $P2_1$, with unit cell dimensions a = 74.0 Å, b = 83.5 Å, c = 38.4 Å, and β = 99.55°. There is one TIM dimer of overall molecular weight 56 000 in the asymmetric unit. Data to 1.9-Å resolution were collected by single-crystal diffractometry and reduced by standard methods (Lolis et al., 1990), with the exception that a reciprocal-space-dependent tensor was fit to the individually measured backgrounds for each reflection to improve the quality of the high-resolution data (Davenport et al., in preparation). The structure of the TIM–PGH complex was solved by a combination of isomorphous replacement and molecular replacement methods. Briefly, the binding sites of mercury atoms in an ethyl mercury phosphate derivative crystal were located by difference Patterson synthesis at medium resolution. The mercury derivative was not sufficient on its own to solve the structure, but it did allow the known structure of the unliganded enzyme to be positioned in this new unit cell. Since mercury is known to bind to Cys-126 in yeast TIM [see Lolis et al. (1990)], it was assumed that the two mercury sites found from the Patterson function represented one mercury binding to Cys-126 on each subunit. The atomic coordinates for uncomplexed yeast TIM were then placed in the TIM–PGH unit cell so that the Cys-126 sulfur positions overlapped the mercury positions. This positioning solves the translation problem and leaves only an unknown rotational orientation of the dimer about the vector between the two cysteines. To determine this orientation, the model was rotated about the vector in 5-deg increments and the crystallographic R-factor was calculated at low resolution. A deep minimum in the plot of R-factor vs rotation angle identified the correct orientation of the TIM dimer in the TIM–PGH unit cell.

Phases were calculated from the properly oriented TIM model, with coordinates for the inhibitor and the side chains of the important active-site residues omitted. These phases were applied to the measured reflection amplitudes from the TIM–PGH crystal to give a difference electron density map of the complex. This map clearly showed the intermediate analogue bound in the active site, clear positions for the active-site side chains, and a large region of negative and positive electron density indicative of the movement of the flexible loop from its "open" position in the model structure to the "closed" position observed in the binding of substrate and transition-state analogue (Lolis & Petsko, 1990; Joseph et al., 1990). Inhibitor coordinates were added and the atomic model was then rebuilt to fit the density. The TIM–PGH structure has now been refined by a combination of restrained least-squares and simulated annealing (Brunger et al., 1987) at 1.9-Å resolution to an R-factor of 18.3% with good stereochemistry. The precision of the final model can be assessed by superposition of the two subunits in the asymmetric unit, giving an rms deviation between α-carbon coordinates of 0.4 Å. Details of the structure solution and the refinement will be published separately (Davenport et al., in preparation).

Results and Discussion

The configuration of the bound reactive intermediate analogue was clear and unambiguous in the initial difference electron density map, for which no information about anything bound to the active site had been included in the phase calculation, as well as in the final electron density map after refinement (Figure 3a). Figures 3b and 4 show the interactions made by the inhibitor with the active-site residues. The position of PGH overlaps the position of DHAP in the medium-resolution Michaelis complex structure (Alber et al., 1991), suggesting that the interactions observed at high resolution for TIM–PGH are likely to be the same as those in the productive enzyme–substrate or enzyme–intermediate complex.

The conformation of Glu-165 in TIM–PGH is significantly different from that observed in the unliganded native yeast and chicken TIM crystal structures (Banner et al., 1975; Lolis et al., 1990). In the free enzyme, the carboxylate is almost completely exposed to solvent. In the TIM–PGH complex, inhibitor binding induces a change in carboxylate orientation. The side chain moves by 2–3 Å from its position in the uncomplexed enzyme, and in the process a hydrogen bond from the carboxylate to the hydroxyl of Ser-96 is replaced by interactions of the carboxylate oxygens with the inhibitor (Figure 4). As is the case with DHAP, PGH binding also causes closure of the flexible loop, resulting in sequestration of the carboxylate from bulk solvent. A bound water molecule and the hydroxyl oxygen of PGH are the only polar groups within hydrogen-bonding distance of Oε1 of the carboxylate, and only the hydroxamate nitrogen is within hydrogen-bonding distance of Oε2 (Figures 3 and 4). Although disordered water molecules cannot be seen in electron density maps, their presence in the active site after substrate is bound would seem to be ruled out on steric considerations, as there is no space large enough to accommodate them in the tightly packed active site after the loop has closed.

This nonaqueous environment should increase the basicity of Glu-165 over its value in the uncomplexed enzyme. The exact magnitude of the effect is hard to estimate, but a qualitative discussion is possible. Glutamic acid in simple

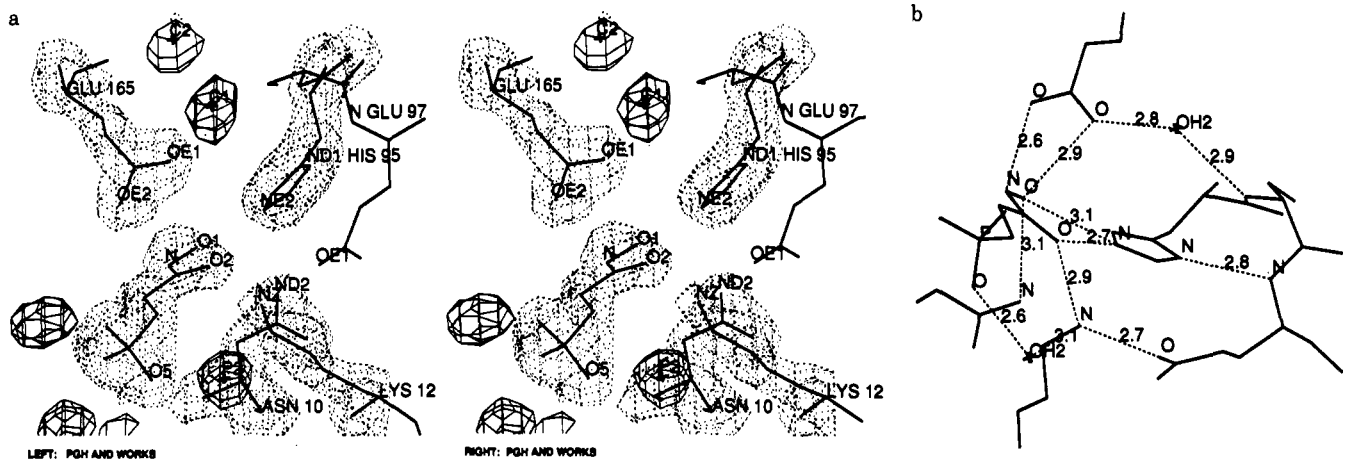

FIGURE 3: (a) Stereo drawing of the atomic coordinates and electron density in the final $F_o - F_c$ map for the refined structure of the TIM–PGH complex. Those residues with associated density were omitted from the phase calculation. The loop of 10 residues (168–177) that folds down over the phosphate group when PGH binds has been excluded from the figure for clarity. (b) Schematic of the active site of the TIM–PGH structure in approximately the same orientation as the stereo diagram. The interatomic distances between potential hydrogen-bond donor and acceptor atoms in the final refined atomic coordinates are indicated in angstroms. The standard deviation of these distances is estimated to be about 0.2 Å.

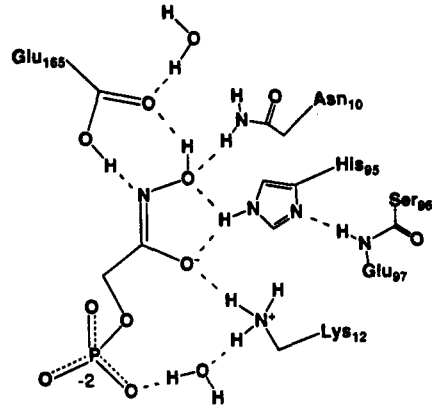

FIGURE 4: Schematic of the active site of yeast triosephosphate isomerase in the complex with PGH. The hydrogen bonds between the active-site residues and the inhibitor are indicated by dotted lines. Also shown are the positions of two bound solvent molecules: one hydrogen-bonded to Glu-165 and the other hydrogen-bonded to the phosphate group of the inhibitor. Note that the main-chain amide of Glu-97 is donating a hydrogen bond to the δ-nitrogen of His-95, so the imidazole must be neutral in this structure. There are additional interactions between the phosphate oxygens and a loop of 10 amino acids, residues 168–177, which folds down over the active site when PGH binds, but this loop has been omitted for clarity.

peptides has a pK_a of around 4.3, which is close to the value of 4.76 for acetic acid in water. Polar but nonaqueous solvents like methanol and DMSO have been shown to increase the pK_a of a carboxylate by 8 units or more: the pK_a of acetic acid is 9.6 in methanol and 12.6 in DMSO (Cox, 1973; Kemp et al., 1975). Although the conformational change in the flexible loop has excluded bulk solvent from contact with the carboxylate, there is a single trapped water molecule still hydrogen-bonded to one of the carboxylate oxygens (Figures 3 and 4). The close proximity of His-95 and a large number of nearby peptide dipoles should also help to make the microenvironment of the catalytic base at least somewhat polar. Thus, the most reasonable model for the enzymic environment would seem to be a polar but nonaqueous solvent, and so it seems likely that the pK_a of Glu-165 in the enzyme–substrate complex is considerably higher than 4.3. On the basis of the pK_a of acetone in water, the proton to be abstracted probably has a pK_a of close to 18 in the free substrate (Chiang et al., 1984), so the increased basicity of Glu-165 due to solvation by the enzyme and the substrate, as opposed to bulk water, is important for efficient catalysis.

From the refined high-resolution structure of the TIM–PGH complex, it is possible to infer the relative orientations of the carboxylate of Glu-165 and the substrate DHAP in the Michaelis complex with some precision. However, it is not possible to infer the protonation state of the carboxylate in the complex. The inhibitor is oriented so that the *pro-R* hydrogen of DHAP would be transferred to the syn orbital of the carboxylate oxygen if the substrate were bound analogously. The syn orbital is in the plane of the v-shaped CO_2 group and points inwards. Gandour has pointed out that the syn orbital should be orders of magnitude more basic than the anti orbital, which points away from the v-shaped carboxylate (Gandour, 1981). Experimental data from Rebek and associates support this suggestion and indicate that the magnitude of the effect varies with, among other factors, the acidity of the hydrogen that is transferred. They find that, in aqueous methanol, the difference in basicity between the two orbitals is 4–5 pK_a units when an acidic hydrogen is approaching the carboxylate; for a less acidic hydrogen such as might be expected in an enolization reaction the difference is still 1–2 units (Rebek, 1990).

The inhibitor is bound in an extended conformation, with the two catalytically important oxygens cis to one another (Figures 2 and 3). This conformation places the bond between C1 and the *pro-R* hydrogen of DHAP periplanar with the π orbitals of the C2 carbonyl group. Stereoelectronic theory suggests that such an alignment would increase the acidity of the C1 proton (Corey & Sneen, 1955). Protonation states of the intermediate are not shown consistently in Figure 2 to emphasize the point that the exact nature of the intermediate is not known. It may be fully protonated as an enediol phosphate; it may exist as two symmetrical endiolates that differ only in the position of the proton on oxygen. No conclusive evidence for either case exists. If the intermediate is the enediol, the transition states leading to it from either direction should resemble the symmetrical enediolates. The neutral state of His-95 would appear to rule out the third alternative, a fully deprotonated intermediate ion-paired with an imidazolium ion, unless a significant structural rearrangement were to take place.

There are three potential electrophiles in contact with the hydroxamyl oxygens of PGH: the side-chain amide of Asn-10, the ε-amino group Lys-12, and the imidazole N_ϵ of His-95

(Figure 3). Asn-10 interacts with O1, and Lys-12 is hydrogen-bonded to O2. The histidine N_ϵ is positioned midway between the two oxygens, although its orientation favors a more linear hydrogen bond to O2. Glutamate-97 appears to stabilize the orientation of the side chain of Lys-12 via a salt bridge. His-95 is at the N-terminal end of a short α-helix; the helix axis points to the PGH. The charge on Lys-12, the amide dipole of Asn-10, and the local "helix dipole" (Hol et al., 1978; Tidor & Karplus, 1991), as relayed through the imidazole ring, are all positive and are all directed toward the oxygen atoms of the inhibitor. It seems likely that electrostatic stabilization of the charged transition state (and possibly of a charged intermediate if this species is the enediolate rather than the enediol) plays a significant role in the catalytic power of TIM.

However, the structure raises interesting questions about the possible role of His-95 as a general acid to protonate the substrate carbonyl oxygen. The Nϵ is perfectly positioned to transfer a hydrogen to either oxygen with minimal motion, and since it is within hydrogen-bonding distance of a hydrogen-bond acceptor, the PGH carbonyl O2, we assume that this nitrogen is protonated in the complex. When histidine functions as an acid, it ordinarily does so by donating a proton from its cationic imidazolium form (both nitrogens protonated), thereby becoming a neutral imidazole. The nitrogens are equally basic (the pK_a of the δ-nitrogen is 6.15 and that of the ϵ-nitrogen is 6.19) but the tautomeric form in which the δ-nitrogen is protonated predominates by at least 4:1 in histidine-containing peptides (Blomberg et al., 1977). The local environment provided by a protein can greatly alter the tautomeric equilibrium constant, in some cases reversing it (Bachovcin & Roberts, 1978). The crystal structure of the TIM–PGH complex suggests that this has happened to His-95: the Nδ of His-95 is within hydrogen-bonding distance of an obligatory hydrogen-bond donor, the main-chain amide of residue 97. The electron density for both the peptide bond between Ser-96 and Glu-97 and the side chain of His-95 is clear and well-defined in both of the independent subunits in the map. In particular, the carbonyl bump is obvious, indicating that the amide group of the peptide bond, rather than the carbonyl group, is oriented toward the imidazole ring. Thus, the N_δ must be unprotonated, and His-95 must be neutral in the PGH complex and, by extension, in the enzyme–substrate complex as well. If His-95 is to act as an acid in proton transfer to substrate oxygen, a role implied by the results on the His-95 to Gln mutant (Nickbarg et al., 1988; Komives et al., 1991), it must do so as the highly unusual imidazole/imidazolate pair (Figure 2c) instead of the normal imidazolium/imidazole equilibrium (Figure 2b), unless the proton transfer from His to oxygen is concerted with a transfer back from the other oxygen to the histidine. In the concerted mechanism, His-95 would function as a "proton shuttle" between the two oxygen atoms, and the intermediate would always be an enediolate, as shown in Figure 1. Nothing in the structure of the TIM–PGH complex allows us to distinguish between these two possibilities or to explain why an unusual form of His might be required.

In summary, the high-resolution structure of yeast TIM complexed with the reactive intermediate analogue inhibitor PGH defines the geometry of both enzyme and substrate in the catalytically important complexes. The structural data suggest that the catalytic efficiency of this highly evolved enzyme arises from several factors: destabilization of the charged carboxylate form of Glu-165 relative to the protonated form by means of a nonaqueous environment, orientation of the carboxylate so as to use the more basic orbital for proton abstraction, binding of the substrate in a conformation that enhances the reactivity of the proton to be transferred, and stabilization of developing negative charge on the substrate oxygen atoms in the transition state by hydrogen bonding and electrostatic (charge–charge and charge–dipole) interactions. On the importance of simple proximity effects and acid catalysis for the mechanism, the structure is mute. Indeed, it poses a question as to the possibility (let alone the desirability) of acid catalysis by a neutral imidazole. X-ray structural data are also unable to give a quantitative measure of the energetic contributions of any of these factors, or of any specific amino acid, for the stabilization of the reaction intermediates or transition states. Specifically, only qualitative inferences concerning the relative contribution to stabilization by Lys-12, Ser-96, Glu-97, Glu-165, Asn-10, and His-95, as well as the mechanistic consequences of the protonation state of His-95, can be obtained. The accompanying paper (Bash et al., 1991) describes a computational approach to address these issues.

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Registry No. TIM, 9023-78-3; His-95, 71-00-1; Lys-12, 56-87-1; Glu-165, 56-86-0.

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 B 293*, 159–171.

Alber, T. C., Davenport, R. C., Giammona, D. A., Lolis, E., Petsko, G. A., & Ringe, D. (1987) *Cold Spring Harbor Symp. Quant. Biol. LII*, 603–613.

Albery, W. J., & Knowles, J. R. (1976a) *Biochemistry 15*, 5631–5640.

Albery, W. J., & Knowles, J. R. (1976b) *Biochemistry 15*, 5627–5631.

Bachovcin, W. W., & Roberts, J. D. (1978) *J. Am. Chem. Soc. 100*, 8041–8047.

Banner, D. W., et al. (1975) *Nature (London) 255*, 609–614.

Bash, P. A., Field, M. J., Davenport, R., Ringe, D., Petsko, G., & Karplus, M. (1991) Computer Simulation and Analysis of the Reaction Pathway of Triosephosphate Isomerase, *Biochemistry* (following paper in this issue).

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

Blacklow, S. C., & Knowles, J. R. (1990) *Biochemistry 29*, 4099–4108.

Blacklow, S. C., Raines, R. T., Lim, W. A., Zamore, P. D., & Knowles, J. R. (1988) *Biochemistry 27*, 1158–1167.

Blumberg, F., Maurer, W., & Ruterjans, H. (1977) *J. Am. Chem. Soc. 99*, 8149–8159.

Brunger, A. T., Kuriyan, J., & Karplus, M. (1987) *Science 235*, 458–460.

Chiang, Y., Kresge, A. J., & Tang, Y. S. (1984) *J. Am. Chem. Soc. 106*, 460–462.

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

Corey, E. J., & Sneen, R. A. (1955) *J. Am. Chem. Soc. 77*, 2505–2509.

Coulson, A. F. W., Knowles, J. R., Priddle, J. D., & Offord, R. E. (1970) *Nature (London) 227*, 180–181.

Gandour, R. D. (1981) *Bioorg. Chem. 10*, 169–176.

Hall, A., & Knowles, J. R. (1975) *Biochemistry 14*, 4348–4352.
Hartman, F. C. (1970) *Biochem. Biophys. Res. Commun. 39*, 384–388.
Hol, W. G. J., van Duijnen, P. T., & Berendson, H. J. C. (1978) *Nature (London) 273*, 443–446.
Joseph, D., Petsko, G. A., & Karplus, M. (1990) *Science 249*, 1425–1428.
Kemp, D. S., Cox, D. D., & Paul, K. G. (1975) *J. Am. Chem. Soc. 97*, 7312–7318.
Komives, E. A., Chang, L. C., Lolis, E., Tilton, R. F., Petsko, G. A., & Knowles, J. R. (1991) *Biochemistry 30*, 3011–3019.
Lolis, E., & Petsko, G. A. (1990) *Biochemistry 29*, 6619–6625.
Lolis, E., Alber, T. C., Davenport, R. C., Rose, D., Hartman, F. C., & Petsko, G. A. (1990) *Biochemistry 29*, 6609–6618.
Nickbarg, E. B., Davenport, R. C., Petsko, G. A., & Knowles, J. R. (1988) *Biochemistry 27*, 5948–5960.
Petsko, G. A., Davenport, R. C., Frankel, D., & RajBhandary, U. L. (1984) *Biochem. Soc. Trans. 12*, 229–232.
Pompliano, D. L., Peyman, A., & Knowles, J. R. (1990) *Biochemistry 29*, 3186–3194.
Rebek, J., Jr. (1990) *Angew. Chem., Int. Ed. Engl. 29*, 245–255.
Richard, J. P. (1984) *J. Am. Chem. Soc. 106*, 4926–4936.
Rieder, S. V., & Rose, I. A. (1959) *J. Biol. Chem. 234*, 1007–1010.
Rose, I. A. (1982) *Methods Enzymol. 87*, 84–97.
Straus, D., Raines, R. Kawashima, E., Knowles, J. R., & Gilbert, W. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 2272–2276.
Tidor, B., & Karplus, M. (1991) *Biochemistry 30*, 3217–3228.
Waley, S. G., Mill, J. C., Rose, I. A., & O'Connell, E. L. (1970) *Nature (London) 227*, 181.

Computer Simulation and Analysis of the Reaction Pathway of Triosephosphate Isomerase†

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ABSTRACT: A theoretical approach designed for chemical reactions in the condensed phase is used to determine the energy along the reaction path of the enzyme triosephosphate isomerase. The calculations address the role of the enzyme in lowering the barrier to reaction and provide a decomposition into specific residue contributions. The results suggest that, although Lys-12 is most important, many other residues within 16 Å of the substrate contribute and that histidine-95 as the imidazole/imidazolate pair could act as an acid/base catalyst.

Although there have been many discussions of the factors contributing to rate enhancement by enzymes (Fersht, 1985; Jencks, 1975, 1987; Kraut, 1988), our understanding is limited by the lack of detailed information at the molecular level. An enzyme that has been the subject of intensive experimental studies is triosephosphate isomerase (TIM). TIM catalyzes the interconversion of dihydroxyacetone phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP), an essential step in the glycolytic pathway. The TIM-catalyzed reaction is 9 orders of magnitude faster than that catalyzed by acetate ion in aqueous solution (Hall & Knowles, 1975; Richard, 1984). Although a wide range of experiments, including kinetics (Rose, 1962; Herlihy et al., 1976; Albery & Knowles, 1976b), X-ray crystallography (Banner et al., 1975; Alber et al., 1981; Davenport et al., 1991), NMR (Browne et al., 1976) and infrared (Belasco & Knowles, 1980) spectroscopy, and site-specific mutagenesis (Nickbarg et al., 1988), have been applied to TIM, the role of the enzyme in determining the energetics along the reaction path is not fully understood. Glu-165 has been implicated as the catalytic base (Rose, 1962; Herlihy et al., 1976; Hartman, 1970; Waley et al., 1970; de la Mare et al., 1972; Banner et al., 1975; Alber et al., 1981), and a recent crystallographic study (Davenport et al., 1991), together with site-specific mutagenesis (His-95 → Gln) (Nickbarg et al., 1988; Komives et al., 1991), suggests that His-95 also plays an important role in the enzymatic reaction.

To obtain a more detailed knowledge of the mechanism of TIM, theoretical approaches of the type now being widely applied to macromolecules of biological interest (Brooks et al., 1988; Karplus & Petsko, 1990) can be used to supplement the available experimental data. We report a calculation of the TIM reaction path that employs a simulation method (Field et al., 1990) developed for the study of reactions in solution

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