A Metabolic Bypass of the Triosephosphate Isomerase Reaction

Kevin K. Desai and Brian G. Miller*

Department of Chemistry and Biochemistry, 217 Dittmer Laboratory of Chemistry, Florida State University, Tallahassee, Florida 32306-4390

Received June 4, 2008; Revised Manuscript Received June 26, 2008

ABSTRACT: Triosephosphate isomerase (TIM) catalyzes the interconversion of D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, an essential step in glycolytic and gluconeogenic metabolism. To uncover promiscuous isomerases embedded within the *Escherichia coli* genome, we searched for genes capable of restoring growth of a TIM-deficient bacterium under gluconeogenic conditions. Rather than discovering an isomerase, we selected *yghZ*, a gene encoding a member of the aldo-keto reductase superfamily. Here we show that YghZ catalyzes the stereospecific, NADPH-dependent reduction of L-glyceraldehyde 3-phosphate, the enantiomer of the TIM substrate. This transformation provides an alternate pathway to the formation of dihydroxyacetone phosphate.

Promiscuous enzymes catalyze alternative reactions that are generally characterized by k_{cat}/K_m values which are orders of magnitude lower than the catalytic efficiencies of their normal reactions (*1, 2*). The persistence of promiscuity in modern proteomes is intriguing given the high selectivity and efficiency normally associated with contemporary protein catalysts. In this study, we sought promiscuous triosephosphate isomerases (TIMs)[1] because this simple reaction presents several unique challenges to polypeptide catalysts.

TIM catalyzes the interconversion of D-glyceraldehyde 3-phosphate (D-GAP) and dihydroxyacetone phosphate (DHAP), an essential step in the Embden−Meyerhof pathway (*3*). In glycolysis, TIM enables the two triosephosphate products of aldolase to form pyruvate; in gluconeogenesis, TIM ensures a steady supply of both triosephosphates to aldolase. The isomerization reaction proceeds via two sequential proton exchanges, accelerated by TIM to diffusion-limited rates, and is considered a paradigm in proton transfer chemistry (*4*). The TIM active site maintains a substrate conformation that minimizes π-orbital overlap of the double bond in the enediol intermediate and the phosphate π-system, thereby disfavoring β-elimination of the phosphate to produce toxic methylglyoxal (*5, 6*). TIM is the archetypal member of the $(\beta/\alpha)_8$-barrel fold enzyme family (TIM barrel), the most common fold among protein catalysts (*7*), thus providing a wealth of potential isomerase scaffolds.

To search for a promiscuous isomerase, we performed a genetic selection (*8*) to uncover genes capable of relieving the metabolic deficiency of a *tpiA*$^-$ strain of *Escherichia coli* (*9*) during gluconeogenic growth. This strain was transformed with a plasmid-borne genomic expression library composed of *E. coli* genes (*10*) and challenged for growth on L-lactate minimal medium. During growth on L-lactate, *E. coli* catalyzes a series of metabolic conversions that lead to the formation of D-GAP. In the presence of a functional TIM, D-GAP is efficiently isomerized to DHAP. Without TIM activity, gluconeogenesis is blocked at D-GAP. Thus, following transformation with the genomic library, *tpiA*$^-$ cells were plated on M9 minimal medium containing L-lactate (2 mM), appropriate antibiotics, and IPTG (50 μM) to stimulate protein production. Colonies displaying a growth advantage over parental control cells appeared after 2 and 4 days of incubation at 37 °C.

Sequencing of plasmid DNA from the selected colonies revealed *tpiA* (fast growing colonies) and *yghZ* (slow growing colonies) as the only genomic library members capable of complementing the metabolic deficiency of the *tpiA*$^-$ strain. The growth advantage provided by *yghZ* overexpression was most apparent when the selection experiments were performed at a high cell density ($\sim 5 \times 10^5$ cfu on a 100 mm × 15 mm plate). High cell densities are known to provide *E. coli* with greater resistance to methylglyoxal, a toxic byproduct of triosephosphate metabolism (*6*). YghZ has been previously shown to possess weak, NADPH-dependent reductase activity toward a variety of small molecule aldehydes (*11, 12*).

Spectrophotometric assays revealed that YghZ does not possess TIM activity. A highly purified preparation of YghZ was incubated with DHAP, and its ability to promote isomerization was assayed by linking the production of D-GAP to NAD^+ reduction via D-glyceraldehyde 3-phosphate dehydrogenase (*13*). No evidence of isomerization was observed during prolonged incubations in the presence of high concentrations of YghZ (50 μM). These experiments place an upper limit of 0.05 M^{-1} s^{-1} on the k_{cat}/K_m value of triosephosphate isomerization. On the basis of these results, we concluded that YghZ does not complement the metabolic deficiency of *tpiA*$^-$ cells by acting as a latent isomerase.

Although inactive as an isomerase, YghZ does function as a triosephosphate reductase. Incubation of YghZ with NADPH and commercially available D,L-GAP resulted in a rapid decrease in cofactor absorbance at 340 nm. ^{1}H NMR of the reaction product established that YghZ catalyzes the NADPH-dependent formation of glycerol 3-phosphate from racemic GAP (Figure S1). YghZ was unable to use NADH

* To whom correspondence should be addressed. Phone: (850) 645-6570. Fax: (850) 644-8281. E-mail: miller@chem.fsu.edu.

[1] Abbreviations: TIM, triosephosphate isomerase; *tpiA*, triosephosphate isomerase gene; D-GAP, D-glyceraldehyde 3-phosphate; L-GAP, L-glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; L-G3P, L-glycerol 3-phosphate; NAD(P)H, nicotinamide adenine dinucleotide (phosphate), reduced form; $NAD(P)^+$, nicotinamide adenine dinucleotide (phosphate), oxidized form; IPTG, isopropyl β-D-thiogalactopyranoside; cfu, colony forming units; AKR, aldo-keto reductase; ^{1}H NMR, proton nuclear magnetic resonance.

10.1021/bi801054v CCC: $40.75
Published on Web 07/12/2008

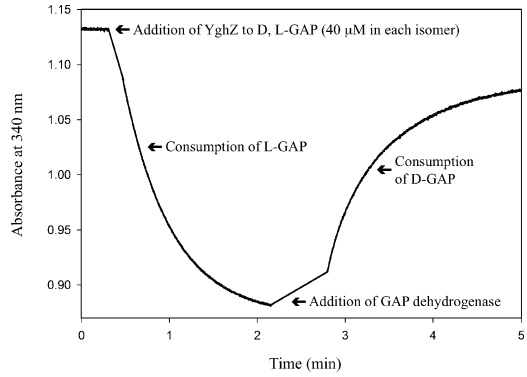


FIGURE 1: Spectrophotometric trace of cofactor absorbance demonstrating that YghZ is specific for L-GAP. The substrate for glyceraldehyde 3-phosphate dehydrogenase, D-GAP, is retained after the YghZ reaction.

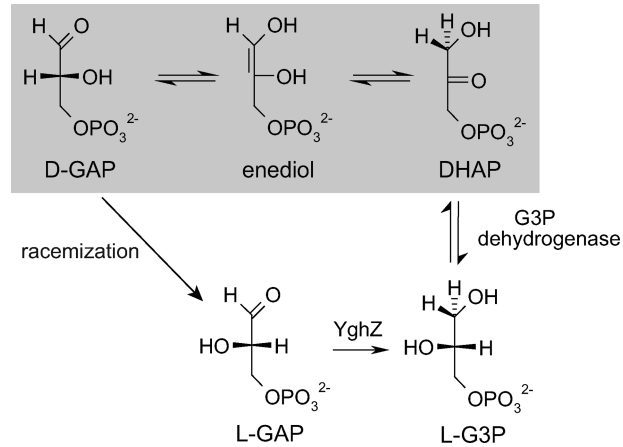
as a cofactor and was inactive toward DHAP. Assays of YghZ conducted in the presence of limiting amounts of D,L-GAP indicated that only 50% of the total aldehyde was consumed, suggesting that only one enantiomer of this triosephosphate is a competent substrate for enzymatic reduction.

Enantiomeric specificity studies demonstrated that YghZ catalyzes the stereospecific reduction of L-GAP. A racemic mixture of GAP was incubated with D-glyceraldehyde 3-phosphate dehydrogenase, and the depletion of D-GAP was monitored by following the increase in absorbance at 340 nm as NAD^+ is reduced. Upon completion of this reaction, YghZ and NADPH were added to the mixture. A rapid decrease in the magnitude of the absorbance signal was observed, demonstrating that the YghZ substrate persisted in the reaction mixture. In the reverse experiment, we observed full retention of D-GAP in the reaction mixture following treatment with YghZ and NADPH (Figure 1). The stereochemical identity of the YghZ product was confirmed to be the L-enantiomer of glycerol 3-phosphate (L-G3P) via observation of its reactivity with L-G3P dehydrogenase. We were unable to detect $NADP^+$-dependent oxidation of L-G3P by YghZ.

The magnitude of the second-order rate constant of enzymatic L-GAP reduction is 4.2×10^5 M^{-1} s^{-1}, suggesting that this reaction may be the physiological function of YghZ. Kinetic analysis revealed a K_m value for L-GAP of 53 μM, a k_{cat} value of 22 s^{-1}, and a K_m value for NADPH of 30 μM (Figure S2). The reactivity of YghZ with L-GAP was significantly stabilized by the inclusion of bovine serum albumin (5 mg/mL) in assay mixtures, which also contained imidazole (0.1 M, pH 6.5) and NADPH (0.25 mM). Previously, Tropp and co-workers have observed that *E. coli* can efficiently incorporate L-GAP into phospholipids by first reducing it to the phospholipid precursor L-G3P (*14*). The enzyme responsible for this reduction was partially purified, was found to be NADPH-dependent, and displayed a K_m value for L-GAP similar to that reported herein. We postulate that YghZ is the source of this previously reported L-GAP reductase activity.

What is the in vivo source of the YghZ substrate L-GAP? L-GAP is not considered a natural metabolite and has never been detected in cellular systems. Under our selection conditions, which utilize the three-carbon acid L-lactate as a sole carbon source, we hypothesize that L-GAP is formed

Scheme 1: Reaction Catalyzed by Triosephosphate Isomerase Bypass[a]



[a] TIM reaction (shaded) proceeds through an enediol. L-GAP may be formed via nonenzymatic or enzymatic racemization of D-GAP. YghZ catalyzes the stereospecific reduction of L-GAP to form L-glycerol 3-phosphate (L-G3P), thus providing an alternate path to DHAP and a metabolic bypass of the TIM reaction.

via nonenzymatic and/or enzymatic racemization of D-GAP that accumulates as a result of the metabolic block in the *tpiA*$^-$ strain. Triosephosphates are highly susceptible to loss of α-carbonyl protons to solvent, forming the enediol depicted in Scheme 1 (*15, 16*). Once generated, this intermediate can spontaneously and nonstereospecifically reprotonate at C1 to form D- or L-GAP, reprotonate at C2 to form DHAP, or collapse to generate methylglyoxal and phosphate. The rate of nonenzymatic racemization of GAP has been determined in vitro to be 1.1×10^{-5} s^{-1} at 30 °C and pH 7.0 (*15*). Although the nonenzymatic formation of L-GAP is very slow, the ability of cells to use this molecule, via expression of YghZ, would nonetheless provide a growth advantage. An enzyme-catalyzed racemization of D-GAP could provide an alternate source of L-GAP, although this enzyme is seemingly very inefficient since *tpiA*$^-$ cells harboring a plasmid-borne *yghZ* gene still grow considerably more sluggishly on L-lactate than do wild-type cells, consistent with growth being limited by L-GAP formation.

We postulate the following mechanism for how YghZ complements a TIM deficiency (Scheme 1). The *tpiA*$^-$ cells, challenged for growth on L-lactate, accumulate D-GAP as a result of the metabolic block at the TIM reaction. The racemization of this metabolite, via nonenzymatic or enzymatic processes, forms L-GAP. The expression of YghZ, resulting from its plasmid-borne derepression, enables the rapid reduction of L-GAP to form L-G3P. Oxidation of L-G3P via constitutively produced L-G3P dehydrogenase (*17*) forms the second TIM product, DHAP, thereby providing a metabolic bypass that allows completion of gluconeogenesis.

YghZ is a member of the aldo-keto reductase (AKR) superfamily. AKRs adopt the $(\beta/\alpha)_8$-barrel fold and reduce various aldehydes and ketones using NAD(P)H as a cofactor (*18*). The true physiological function of most AKRs is unknown due to their broad and overlapping substrate specificities. Metabolically, AKRs are hypothesized to act as detoxifying enzymes that prevent the accumulation of reactive aldehydes and ketones, which can result in the undesirable modification of cellular macromolecules. YghZ

maintains four highly conserved active site residues (Tyr, His, Lys, and Asp), with tyrosine functioning as the putative catalytic acid. Indeed, replacement of the active site tyrosine of YghZ with phenylalanine abolished L-GAP reductase activity. Interestingly, the closest mammalian homologue of YghZ is the β-subunit of voltage-gated potassium channels (*19*). The β-subunit is an AKR that forms a permanent complex with the potassium channel and modifies its function but has no known substrate.

YghZ is known to possess a low level of methylglyoxal reductase activity, which forms the dead-end metabolite acetol (*11, 12*). The methylglyoxal reductase activity of YghZ is characterized by a k_{cat}/K_m value that is more than 2 orders of magnitude lower than the L-GAP reductase activity reported herein. It is possible that overexpression of YghZ confers limited resistance to methylglyoxal and that the growth advantage afforded by YghZ may be the combined result of L-GAP reduction and methylglyoxal detoxification. The failure to select other known, more efficient methylglyoxal detoxifying enzymes, despite their presence in the genomic library, suggests that the primary function of YghZ in our selection is to reduce L-GAP.

L-GAP is a potent toxin, partially due to the close structural resemblance that its hydrated form bears to the phospholipid precursor L-G3P (*20*). L-GAP is known to inhibit two enzymes involved in phospholipid biosynthesis, L-glycerol-3-phosphate acyltransferase and phosphatidylglycerol phosphate synthase (*20*), as well as the glycolytic enzyme aldolase (*21*). Given the potentially hazardous consequences of L-GAP formation and the high degree of evolutionary conservation of *yghZ*, we postulate that Nature has evolved YghZ as a mechanism for the detoxification of L-GAP formed nonenzymatically or by other unknown cellular processes. Efficient pathways have been established to detoxify methylglyoxal, the elimination product of triosephosphates, via glyoxalase-dependent metabolism to D-lactate (*6*). Our findings provide the first evidence of a pathway for L-GAP removal.

The ability of metabolic bypasses to compensate for deficiencies in essential enzymes has been previously described (*22*), and our report further illustrates the robustness of the *E. coli* metabolic network. In addition, our results suggest that systematic searches for promiscuous catalysts may assist in the assignment of function to uncharacterized genes (*23*).

ACKNOWLEDGMENT

We thank Professor Liz Hedstrom for suggesting the possibility that L-GAP forms via D-GAP racemization.

SUPPORTING INFORMATION AVAILABLE

Experimental methods, Michaelis−Menten plots, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. O'Brien, P. J., and Herschlag, D. (1999) Catalytic promiscuity and the evolution of new enzyme activities. *Chem. Biol. 6*, R91–R105.
2. Jensen, R. A. (1976) Enzyme recruitment in the evolution of new function. *Annu. Rev. Microbiol. 30*, 409–425.
3. Meyerhof, O., and Junowicz-Kocholaty, R. (1943) The equilibria of isomerase and aldolase, and the problem of the phosphorylation of glyceraldehyde phosphate. *J. Biol. Chem. 149*, 71–92.
4. Knowles, J. R., and Albery, W. J. (1977) Perfection in enzyme catalysis: The energetics of triosephosphate isomerase. *Acc. Chem. Res. 10*, 105–111.
5. Lolis, E., and Petsko, G. A. (1990) Crystallographic analysis of the complex between triosephosphate isomerase and 2-phosphoglycolate at 2.5-Å resolution: Implications for catalysis. *Biochemistry 29*, 6619–6625.
6. Cooper, R. A. (1984) Metabolism of methylglyoxal in microorganisms. *Annu. Rev. Microbiol. 38*, 49–68.
7. Reardon, D., and Farber, G. K. (1995) The structure and evolution of α/β barrel proteins. *FASEB J. 9*, 497–503.
8. Miller, B. G., and Raines, R. T. (2004) Identifying latent enzyme activities: Substrate ambiguity within modern bacterial sugar kinases. *Biochemistry 43*, 6387–6392.
9. Kang, Y., Durfee, T., Glasner, J. D., Qiu, Y., Frisch, D., Winterberg, K. M., and Blattner, F. R. (2004) Systematic mutagenesis of the *Escherichia coli* genome. *J. Bacteriol. 186*, 4921–4930.
10. Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H., and Mori, H. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library: Unique resources for biological research. *DNA Res. 12*, 291–299.
11. Grant, A. W., Steel, G., Waugh, H., and Ellis, E. M. (2003) A novel aldo-keto reductase from *Escherichia coli* can increase resistance to methylglyoxal toxicity. *FEMS Microbiol. Lett. 218*, 93–99.
12. Ko, J., Kim, I., Yoo, S., Min, B., Kim, K., and Park, C. (2005) Conversion of methylglyoxal to acetol by *Escherichia coli* aldo-keto reductases. *J. Bacteriol. 187*, 5782–5789.
13. Plaut, B., and Knowles, J. R. (1972) pH-dependence of the triose phosphate isomerase reaction. *Biochem. J. 129*, 311–320.
14. Kalyananda, M. K., Engel, R., and Tropp, B. E. (1987) Metabolism of L-glyceraldehyde 3-phosphate in *Escherichia coli*. *J. Bacteriol. 169*, 2488–2493.
15. Hall, A., and Knowles, J. R. (1975) The uncatalyzed rates of enolization of dihydroxyacetone phosphate and of glyceraldehyde 3-phosphate in neutral and aqueous solution. The quantitative assessment of the effectiveness of an enzyme catalyst. *Biochemistry 14*, 4348–4352.
16. Richard, J. P. (1984) Acid-base catalysis of the elimination and isomerization reactions of triose phosphates. *J. Am. Chem. Soc. 106*, 4926–4936.
17. Edgar, J. R., and Bell, R. M. (1978) Biosynthesis in *Escherichia coli* of *sn*-glycerol 3-phosphate, a precursor of phospholipid. *J. Biol. Chem. 253*, 6348–6353.
18. Ellis, E. M. (2002) Microbial aldo-keto reductases. *FEMS Microbiol. Lett. 216*, 123–131.
19. Gulbis, J. M., Mann, S., and MacKinnon, R. (1999) Structure of a voltage-dependent K^+ channel β subunit. *Cell 97*, 943–952.
20. Tang, C. T., Engel, R., and Tropp, B. E. (1977) L-Glyceraldehyde 3-phosphate, a bactericidal agent. *Antimicrob. Agents Chemother. 11*, 147–153.
21. Leoncini, G., Maresca, M., Ronchi, S., and Bonsignore, A. (1979) Comparative aspects of the inactivation of fructose 1,6 bisphosphate aldolase by D- and L-glyceraldehyde 3-phosphate. *Ital. J. Biochem. 28*, 232–244.
22. D'Ari, R., and Casadesus, J. (1998) Underground Metabolism. *BioEssays 20*, 181–186.
23. Keseler, I. M., Collado-Vides, J., Gama-Castro, S., Ingraham, J., Paley, S., Paulsen, I. T., Peralta-Gil, M., and Karp, P. D. (2005) EcoCyc: A comprehensive database resource for *Escherichia coli*. *Nucleic Acids Res. 33*, D334–D337.

BI801054V