Point of VIEW

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Restoring a Metabolic Pathway

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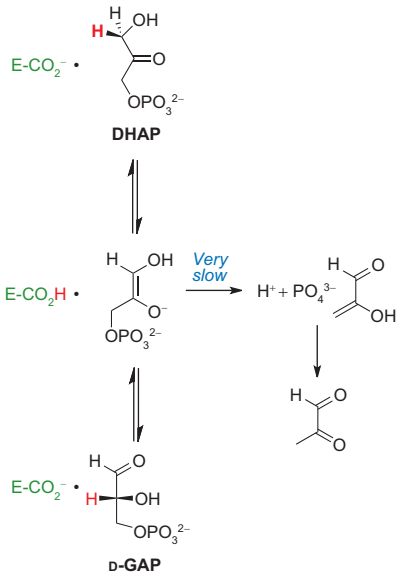
ABSTRACT Gluconeogenesis is blocked in a strain of *Escherichia coli* that is deficient in triosephosphate isomerase, but it was restored by the insertion of a plasmid coding for an L-glyceraldehyde 3-phosphate reductase (YghZ). This reductase provides a "bypass" that produces dihydroxyacetone phosphate (DHAP) by the consecutive enzyme-catalyzed reduction of L-glyceraldehyde 3-phosphate (L-GAP) by NADPH to give L-glycerol 3-phosphate and reoxidation by NAD^+ catalyzed by endogenous L-glycerol 3-phosphate dehydrogenase to give DHAP. The origin of cellular L-GAP remains to be determined.

The study of metabolic pathways, perhaps the most mature of all areas of experimental biochemistry, has led to many important discoveries. This work has demonstrated a powerful elegance to the chemical logic of metabolic pathways and has provided biochemists with many important examples of how the trial and error of evolution leads to simple solutions to inconceivably complex problems. Opportunities for new discovery in mature areas of research will generally only be presented when original questions are asked and novel approaches are adopted to address these questions. This is illustrated in the work of Desai and Miller (*1*), who created a metabolic bypass in a strain of *Escherichia coli* in which gluconeogenesis is blocked by deletion of the gene coding for triosephosphate isomerase (TIM).

TIM catalyzes the reversible and stereospecific 1,2-hydrogen shift at D-glyceraldehyde 3-phosphate (D-GAP) to form dihydroxyacetone phosphate (DHAP) by a single base (Glu165) proton transfer mechanism through an enzyme-bound *cis*-enediolate phosphate intermediate (Scheme 1) (*2*). The enzyme-bound enediolate also undergoes very slow competing β-elimination of phosphate to form methylglyoxal (*3*). TIM is required for both the breakdown of glucose *via* glycolysis and its biosynthesis *via* gluconeogenesis. *E. coli* strain DF502 is deficient in TIM as a result of a deletion mutation. This strain does not grow on plates with a minimal medium that contains either L-lactate or glycerol as the sole carbon source, but its growth is supported on a medium that contains both of these compounds (*4*). This is because L-lactate is metabolized to D-GAP and glycerol to DHAP, and these compounds are substrates for the aldolase-catalyzed formation of fructose 1,6-bisphosphate. The sugar bisphosphate then moves further through the gluconeogenesis pathway to form glucose (Figure 1). The DF502 strain has been widely utilized for the overexpression of site-directed mutants of TIM with low catalytic activity (*5*).



Scheme 1.

In their study of the TIM-deficient (*tpiA*$^-$) *E. coli* strain FB21547 (*6*), Desai and Miller (*1*) asked the following: "Does the *E. coli* genome code for a protein that is capable of restoring the growth of TIM-deficient *E. coli* on a minimal medium with L-lactate as the sole carbon source?" TIM is the titular mem-

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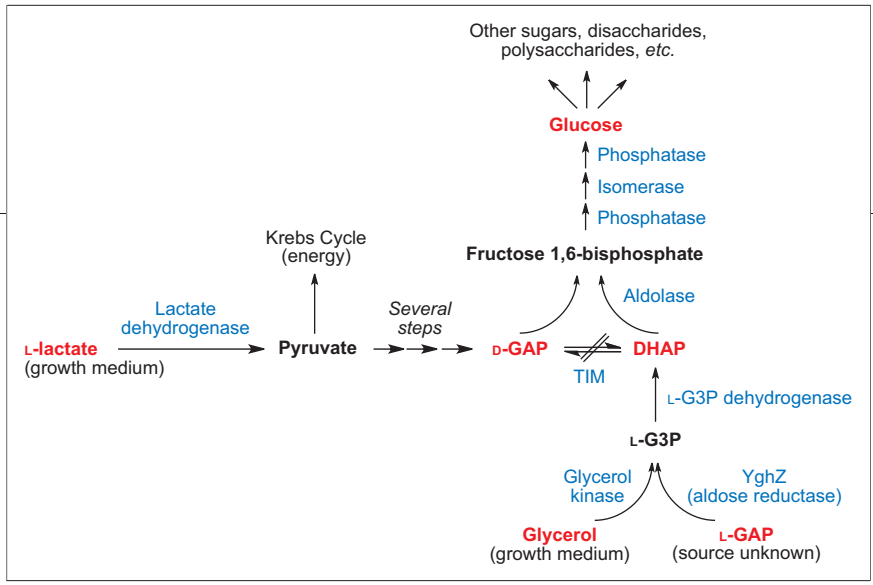

Figure 1. Metabolism of L-lactate and L-GAP *via* gluconeogenesis to form glucose in cells that are deficient in TIM. These cells do not grow on a minimal medium that contains only L-lactate or glycerol, because they are unable produce *both* D-GAP and DHAP, which are required for the production of glucose *via* gluconeogenesis. This pathway is restored in cells grown on L-lactate by insertion of a plasmid coding for the L-GAP reductase YghZ.

ber of the very large group of enzymes with $(\beta/\alpha)_8$-barrel ("TIM-barrel") folds (*7*), which may be the most common of protein folds. The driving hypothesis for Desai and Miller's work was that other evolutionarily related TIM-barrel enzymes from the *E. coli* genome might show promiscuity (*8, 9*) for catalysis of the isomerization of triose phosphates that is sufficient to support the gluconeogenic growth of TIM-deficient *E. coli* on L-lactate. The isolation and the structural and mechanistic characterization of such promiscuous TIM activities have the potential to provide insight into the evolution of protein function. Furthermore, an examination of a family of proteins that catalyze the isomerization of triose phosphates might serve to define the minimal common structural elements required for enzymatic catalysis. These elements could then be incorporated in efforts toward the *de novo* design of proteins with TIM activity.

Aside from TIM itself, the overexpression of just a single member of a plasmid-borne genomic expression library composed of *E. coli* genes (*10*), *yghZ*, complemented the metabolic deficiency of the *tpiA*$^-$ strain. There are no proteins in the *E. coli* genome with sufficient promiscuous TIM activity to complement this deficiency, because the protein product of *yghZ* shows no detectable activity toward isomerization of DHAP. Apparently, TIM is not only a perfect enzyme (*2*), but it also catalyzes a specialized reaction that is not observed as a promiscuous activity for other enzymes in the *E. coli* genome. This is consistent with a large degree of divergence of TIM from its evolutionary precursor.

The gene product, YghZ, is a protein that was previously reported to show a weak NADPH-dependent activity for the reduction of a variety of low-molecular-weight aldehydes (*11*). Desai and Miller showed that YghZ also catalyzes the stereospecific reduction of L-glyceraldehyde 3-phosphate (L-GAP) by NADPH to give L-glycerol 3-phosphate (L-G3P) and $NADP^+$ (Figure 1). The subsequent reoxidation of L-G3P to give DHAP catalyzed by L-glycerol 3-phosphate dehydrogenase then completes the isomerization of L-GAP to give the DHAP that is required to complement the TIM deficiency in the *tpiA*$^-$ strain and restore its ability to grow on L-lactate (Figure 1).

The second-order rate constant $k_{cat}/K_m = 4.2 \times 10^5\ M^{-1}\ s^{-1}$ for enzymatic reduction of L-GAP catalyzed by YghZ in the presence of a saturating concentration of NADPH is similar to $k_{cat}/K_m = 1.0 \times 10^6\ M^{-1}\ s^{-1}$ reported for the reduction of DHAP by NADH catalyzed by L-glycerol 3-phosphate dehydrogenase (*12*). This supports the conclusion that YghZ functions physiologically as a L-GAP reductase to convert L-GAP to L-G3P.

Desai and Miller's combination of well-designed experiments and a thoughtful hypothesis has produced significant results, even in the absence of any evidence to support the original hypothesis that the vast array of $(\beta/\alpha)_8$-barrel proteins within the *E. coli* genome may include enzymes with a promiscuous TIM activity. The work raises the additional question of the origin of cellular L-GAP, the substrate in the proposed metabolic bypass of TIM. L-GAP might form as the product of an aldolase-catalyzed cleavage of a larger sugar phosphate or of a cleavage reaction catalyzed by an enzyme that uses thiamine pyrophosphate as a cofactor. However, no such enzymes have been identified within the *E. coli* genome.

It was previously observed that cell-free extracts of *E. coli* catalyzed an NADPH-dependent reduction of L-GAP to L-G3P (*13*). Desai and Miller raise the possibility that L-GAP arises mainly from the nonenzymatic racemization of D-GAP. D-GAP undergoes deprotonation to form an enediolate phosphate intermediate with a half-time of ~2 h at neutral pH and 37 °C (*3*). Buffer catalysis of this reaction is weak at pH 7, because Brønsted bases do not compete well with the facile intramolecular deprotonation of the C-2 carbon of D-GAP by the substrate phosphate dianion group (*3*). Once formed, the enediolate intermediate partitions between expulsion of phosphate dianion to form methylglyoxal (99% yield) and nonstereospecific reprotonation at C-1 to give the isomerization product DHAP (~0.8% yield) and at C-2 to give the racemization product L-GAP (~0.2% yield) (Figure 2) (*3*).

The propensity of the enediolate phosphate intermediate of the TIM-catalyzed reaction to break down to form the toxic product methylglyoxal is the least elegant feature of glycolysis (*14*). The evolution or *de novo* design of a protein catalyst of the isomerization of D-GAP by a proton transfer mechanism might be expected to result instead in a methylglyoxal synthase (*15*). Nature has done a good job of directing the partition-

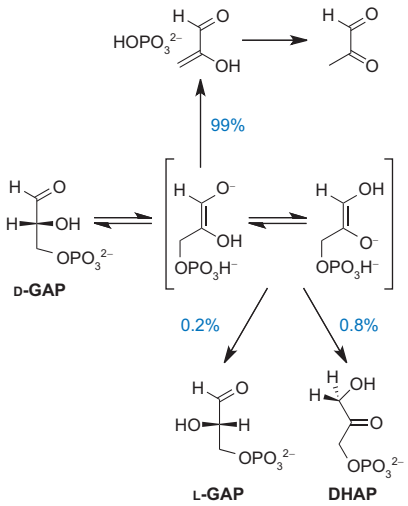

Figure 2. Relative yields of the products from partitioning of the enediolate phosphate intermediate of the *nonenzymatic* reaction of D-GAP in water at pH 7 in the presence of low concentrations of buffer. Intramolecular proton transfer to the substrate phosphate dianion group results in an intermediate that partitions between β-elimination of phosphate to give methylglyoxal (99%), reprotonation at C-1 to form DHAP (0.8%), and reprotonation at C-2 to form L-GAP (0.2%) (*3*).

ing of the enediolate intermediate at the active site of TIM toward the isomerization products and the cell does a good job of removing methylglyoxal in reactions catalyzed by glyoxalases 1 and 2 (*14*). By contrast, in the absence of cellular enzymes to remove L-GAP, the slow nonenzymatic racemization of D-GAP to L-GAP would result in the accumulation of L-GAP. Desai and Miller suggest that the physiological function of YghZ may be to prevent the accumulation of L-GAP that is formed mainly by the nonenzymatic reaction of D-GAP (Figure 2).

It is arguable whether the major pathway for the formation of cellular L-GAP is the nonenzymatic isomerization of D-GAP. This is because it is not clear why an enzyme that catalyzes the reduction of L-GAP to give L-G3P would complement a TIM deficiency, if L-GAP were produced slowly, in a nonenzymatic reaction. First, it is not clear that a very slow nonenzymatic pathway would be competent to support gluconeogenesis, where all of the other reactions are enzyme catalyzed. Second, the nonenzymatic formation of DHAP from D-GAP at pH 7 (Figure 2) is ~4-fold faster than the formation of L-GAP (*3*). Therefore, such a hypothetical TIM bypass would result in only a 25% increase in the overall rate of formation of DHAP. A simple explanation for these results is that there is an enzymatic pathway for the formation of L-GAP that generates the larger quantities of this compound that may be needed to support gluconeogenesis in the TIM-deficient strain of *E. coli* that contains high levels of YghZ.

One goal of chemical biology is to manipulate metabolic pathways in bacteria in order to produce significant quantities of therapeutic agents and industrially useful compounds. The serendipitous observation of a metabolic bypass for triosephosphate isomerase was not directed toward the specific goal of pathway design. However, it makes the important point that there is a flexibility in the performance of these pathways, which may be useful to remember when trying to solve problems encountered in their design.

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