Biosynthesis in *Escherichia coli* of sn-Glycerol-3-Phosphate, a Precursor of Phospholipid

FURTHER KINETIC CHARACTERIZATION OF WILD TYPE AND FEEDBACK-RESISTANT FORMS OF THE BIOSYNTHETIC sn-GLYCEROL-3-PHOSPHATE DEHYDROGENASE*

(Received for publication, June 11, 1979, and in revised form, December 11, 1979)

J. Ralph Edgar and Robert M. Bell‡

From the Department of Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Homogeneous wild type and feedback-resistant forms of the biosynthetic glycerol-3-phosphate dehydrogenase (NAD⁺) of *Escherichia coli* (EC 1.1.1.8) were employed for studies of substrate and inhibitor specificity. The phosphonate analog of dihydroxyacetone-P, 4-hydroxy-3-oxybutyl 1-phosphonate, and glycolaldehyde phosphate proved to be substrates of both enzymes. NADPH, NADH, and nicotinamide hypoxanthine dinucleotide were used as substrates about equally well by both enzymes. Both enzymes were inhibited to the same degree by a number of compounds which were competitive inhibitors with respect to NADPH. The enzymes were shown to have B-type stereospecificity for NADPH. All of these kinetic characterizations indicate that the active sites of the two enzymes are virtually identical. The phosphonate analog of glycerol-P, 3,4-dihydroxybutyl 1-phosphonate, resembled glycerol-P in that it was a competitive inhibitor with respect to dihydroxyacetone-P and its Ki was greater than 10-fold higher for the feedback-resistant than the wild type glycerol-P dehydrogenase. The Ki values for ethylene glycol-P were similar (about 1.4 mM) for both enzymes. The Kr for the ethylene glycol-P was similar to the Km for glycolaldehyde-P for both enzymes, a result unlike any other substrate-product pair tested where Km values were 20- to 40-fold and 1.5- to 3.7-fold higher than Kr values for the wild type and feedback-resistant enzymes, respectively. An interpretation of these data is that glycerol-P and its phosphonate analog inhibit at a glycerol-P regulatory site while ethylene glycol-P inhibits by interaction at the active site. The kinetic properties of the feedback-resistant and wild type glycerol-P dehydrogenases are remarkably similar with respect to catalytic constants but dramatically different with respect to inhibition by glycerol-P and its phosphonate analog. These kinetic data suggest a regulatory site for glycerol-P inhibition.

In *Escherichia coli*, sn-glycerol-3-phosphate (glycerol-P), a precursor of all phospholipid species (1), is synthesized by a soluble enzyme that catalyzes the NAD(P)H-dependent reduction of dihydroxyacetone phosphate (dihydroxyacetone-P) (2). The biosynthetic role for this enzyme in vitro was established by the isolation of glycerol (3) and glycerol-P auxotrophs of *E. coli* (4) deficient in this activity; these strains were unable to synthesize phospholipid in the absence of their required supplements (4). The gpsA locus defined the structural gene for the biosynthetic glycerol-P dehydrogenase (5).

The potent inhibition in vitro of the biosynthetic glycerol-P dehydrogenase by glycerol-P suggested that glycerol-P synthesis in vitro might be regulated by glycerol-P inhibition (2). This mode of regulation was established in vivo by the isolation of mutants, designated *gpsA*RF for feedback-resistant, in which the regulation of glycerol-P synthesis was altered in vivo and which had a glycerol-P dehydrogenase activity having reduced sensitivity in vitro to glycerol-P feedback inhibition (6).

The precise physical and kinetic comparison of the wild type and feedback-resistant glycerol-P dehydrogenases became possible with the extensive purification of the enzymes to homogeneity (7). The wild type and feedback-resistant forms of enzyme were found to have identical physical properties (7). Kinetic analysis in the absence of products demonstrated that both enzymes had identical kinetic mechanisms and identical kinetic constants (8). Analysis of product inhibition revealed a single significant difference in the two enzymes, that the Ki for glycerol-P of the feedback-resistant enzyme was 10-fold that of the wild type (8). This and other data suggested strongly that glycerol-P feedback inhibition occurred at a regulatory site (8). In addition, the properties of inhibition of the wild type glycerol-P dehydrogenase by long chain acyl coenzyme A thioesters in vitro suggested that these compounds might serve in vivo to modulate glycerol-P dehydrogenase activity (9).

In this paper, a number of compounds related structurally to glycerol-P and dihydroxyacetone-P and to NADPH were employed in kinetic investigations of homogeneous wild type and feedback-resistant glycerol-P dehydrogenases. The results provide new insights into glycerol-P synthesis in *E. coli* and its regulation by providing: 1) a description of specificity of each enzyme for substrates and inhibitors; 2) an evaluation of whether other metabolites might regulate enzyme activity in vivo; and 3) additional evidence that glycerol-P inhibition occurs at a regulatory site.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dihydroxyacetone-P dimethylketal, sn-glycerol 3(1)-P, adenosine, 5'-AMP, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, horse liver alcohol dehydrogenase, and glucose 6-phosphate dehydrogenase were purchased from Sigma, NADPH, NADP, NAD, NADH, nicotinamide hypoxanthine dinucleotide, NMN, 2',5'-ADP, ATP, ADP, and ADP-ribose were from P-L Biochemicals. Ribose 5-phosphate, D-erythrose 4-phosphate dimethylacetal, glycolaldehyde 3-phosphate, and dihydroxyacetone-P were from Calbiochem. D-Glycerol-dehyde 3-phosphate and hexokinase were from Boehringer Mann-
Biosynthetic Glycerol-P Dehydrogenase

3493

Heim, and [1-3H]glucose and [1-3H]ethanol were from New England Nuclear. 5,4-Dihydroxybutyl 1-phosphonate and 4-hydroxy-3-oxobuty1-1-phosphonate were the generous gifts of Dr. Burton Tromp, City University of New York. Sulfuric acid was the generous gift of Dr. William Dowhan, University of Texas Medical Center, Houston.

Dihydroxyacetone-P, d-erythrose 4-phosphate, glycolaldehyde phosphate, and glyceraldehyde 3-phosphate were formed from their respective ketals and acetals according to the manufacturers' instructions. L-3-Propanediol phosphate (propanediol-P) was synthesized by the method of Tener (10). Glycerol sulfate was generated from saponification (11). Ethylene glycol phosphate was the method of Tener (10). Glycerol sulfate was generated from sulfatidic acid by saponification (11). Ethylene glycol phosphate was the method of Tener (10). Glycerol sulfate was generated from sulfatidic acid by saponification (11). Ethylene glycol phosphate was the method of Tener (10). Glycerol sulfate was generated from sulfatidic acid by saponification (11). Ethylene glycol phosphate was the method of Tener (10). Glycerol sulfate was generated from sulfatidic acid by saponification (11).

William Dowhan, University of Texas Medical Center, Houston. A-4-3H-NADPH (specific activity, 14,200 dpm/nmol) was purified by gel filtration and DEAE-cellulose (DE52), eluted by ammonium bicarbonate gradient. The product had an A260/A450 ratio of 2.3, was lyophilized, and stored at -80°C in 50 mM Tris-HCl (pH 7.4).

Analysis of Reaction Products—Paper chromatography on Whatman paper in two solvent systems was used to separate glycerol-P dehydrogenase from NADP and NADPH. In Solvent System I, 95% ethanol, 1 M ammonium acetate (pH 7.5) (70:30), NADP, NADPH, and glycerol-P had respective Rf values of 0.045, 0.065, and 0.32. In Solvent System II, propanediol-NH₂·H₂O (60:40:10), NADP, NADPH, and glycerol-P had respective Rf values of 0.16, 0.21, and 0.38. Authentic standard compounds were located by Hanes-Ishcerwood (17) phosphate spray. Chromatograms containing reaction mixtures were cut into 1.0-cm strips and counted in 0.4 ml of water and 4.0 ml of Aquasol II. Greater than 80% of the applied radiolabel was recovered under these conditions.

RESULTS

Substrates Substituted for Dihydroxyacetone-P—In order to probe the specificity of the active sites of homogeneous wild type and feedback resistant glycerol-P dehydrogenases, the enzymes were subjected to two-substrate kinetic analysis for NADPH and 4-hydroxy-3-oxobuty1-1-phosphonate, an analog of dihydroxyacetone-P. Previous work by Cheng et al. (18) demonstrated that 4-hydroxy-3-oxobuty1-1-phosphonate was a substrate for the enzyme and that 3,4 dihydroxybutyl-1-phosphonate was an inhibitor. The plots of v against (4-hydroxy-3-oxobuty1-1-phosphonate) formed straight lines at several, fixed levels of NADPH (Fig. 1, A and C). The family of lines formed at different levels of NADPH intersected near a point on the negative x-axis. These patterns are consistent with a sequential kinetic mechanism. The kinetic constants determined from replots of these data (Fig. 1, B and D) were essentially identical for the wild type and feedback-resistant glycerol-P dehydrogenase (Table I). Plots of v against NADPH at fixed levels of the co-substrate (data not shown) consist of similar patterns of intersecting lines for both forms of glycerol-P dehydrogenase.

These data provide an interesting comparison to that found for dihydroxyacetone-P and NADPH as substrates (8). The maximum velocity (Vmax) of 14 nmol/min/mg is 5-fold lower than that found with dihydroxyacetone-P and NADPH as substrates (Table I). The Michaelis constant (Km) of 0.34 mM for 4-hydroxy-3-oxobuty1-1-phosphonate is 2-fold higher than that for dihydroxyacetone-P, while the Michaelis constant (Km) for NADPH was approximately 4 µM for both substrates. The Km for NADPH was about 5 µM for both enzymes for either dihydroxyacetone-P or the phosphate analog. This is consistent with an ordered kinetic mechanism in which NADPH is the first substrate to bind to free enzyme (13).

Both wild type and feedback-resistant glycerol-P dehydrogenases employed glycolaldehyde phosphate (glycolaldehyde-P) as substrate in the presence of saturating (0.1 mM) NADPH (Fig. 2, A and B). Both enzymes had virtually identical apparent Vmax and Km values, and compared to the kinetic constants found with dihydroxyacetone-P as substrate, Vmax was 6-fold lower and Km was 40-fold higher (Table I).

Dihydroxyacetone and four-, five-, and six-carbon ketose and aldose sugar-phosphates were not substrates at concentrations up to 10 µM at a saturating level of NADPH (0.1 mM). D- and L-glycerol-P and D- and L-glycerol-P dehydroygenases did not appear to be substrates. These data, summarized in Table I, indicate that both wild type and feedback-resistant forms of glycerol-P dehydrogenase are virtually identical in their substrate specificity for dihydroxyacetone-P and related compounds.

Inhibition by Compounds Related to Glycerol-P and Dihydroxyacetone-P—In order to attempt to determine whether glycerol-P inhibition of the glycerol-P dehydrogenase is mediated through a regulatory site and to define the specificity of inhibition, the patterns of inhibition and inhibitor constants were determined for a number of compounds related to glycerol-P and dihydroxyacetone-P. For both forms of glycerol-P dehydrogenase, 3,4-dihydroxybutyl 1-phosphonate, an analog of glycolaldehyde-P, was a potent competitive inhibitor with respect to dihydroxyacetone-P at saturating NADPH (0.1 mM) (Fig. 3). Since the respective K values for the wild type and feedback-resistant enzymes were 15 and 250 µM, the inhibition by this compound resembles that of glycerol-P. However, glycerol-P is a more potent inhibitor than 3,4-dihydroxybutyl 1-phosphonate (Table II). Inorganic phosphate (P) under similar conditions was a weak competitive inhibitor of the wild type and feedback-resistant glycerol-P dehydrogenases (Fig. 4); the respective K values for P of the two enzymes were 2.8 and 8.8 mM.

For wild type and feedback-resistant glycerol-P dehydrogenase, ethylene glycol phosphate (ethylene glycol-P), the product of glycolaldehyde-P reduction, was a competitive inhibitor with respect to dihydroxyacetone-P with saturating NADPH (Fig. 5). The K values for both enzymes were approximately equal (Table II). The substrate/product pair, glycolaldehyde-P/ethylene glycol-P, proved to be an equally good substrate and inhibitor of both the wild type and feedback-resistant enzymes. This behavior was different than all the other substrate/product pairs tested (Table II) where the product K values of the wild type and feedback-resistant enzymes always differed about 10-fold.

The compounds, glycolaldehyde-P and glycolaldehyde-P, were noncompetitive inhibitors of the wild type and feedback-resistant glycerol-P dehydrogenase at saturating NADPH (0.1 mM) (Fig. 6). The K and K values for glycolaldehyde-

1 Figs. 1 to 8 and Tables I to IV are presented in a miniprint supplement at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, Md. 20014. Request Document No. 79111-1156, cite author(s), and include a check or money order for $2.10 per set of photocopies.

2 At the lowest concentration of dihydroxyacetone-P used in these experiments, v was about 13 µmol/min/mg, while the concentrations of glycolaldehyde-P used produced v values less than 1.5 µmol/min/mg in the absence of dihydroxyacetone-P (Fig. 2). Therefore, under these conditions, dihydroxyacetone-P would essentially be the only substrate utilized.
P and glyceraldehyde-P were similar for both the wild type and feedback-resistant glycerol-P dehydrogenases (Table II). The $K_m$ and apparent $K_m$ for glycolaldehyde-P were also similar (compare Tables I and II).

A number of other phosphorylated compounds proved to be competitive inhibitors with respect to dihydroxyacetone-P for the wild type and feedback-resistant glycerol-P dehydrogenases (Table II). Both forms of enzyme had similar $K_m$ values for these compounds.

**Substrates Substituted for NADPH—**Wild type and feedback-resistant glycerol-P dehydrogenases have an apparent $K_m$ for NADPH of about 4 $\mu$m with saturating dihydroxyacetone-P (1.0 mM), in agreement with previously reported values (8). NADH and nicotinamide hypoxanthine dinucleotide were good substrates (Table III). The maximum velocity observed with these compounds was somewhat lower than with NADPH, but their apparent $K_m$ values were similar.

**Inhibition by Analogs of NADPH—**For the wild type and feedback-resistant enzymes, a number of compounds produced competitive inhibition with respect to NADPH at saturating dihydroxyacetone-P (1.0 mM). For all the compounds listed in Table IV, the $K_m$ values were derived from plots similar to that shown in Fig. 7 for ADP-ribose. The wild type enzyme had a $K_m$ essentially identical with that of the feedback-resistant enzyme for each compound tested. NADP, NAD, and ADP-ribose were the most potent inhibitors and produced similar $K_m$ values. These $K_m$ values were 50-fold higher than the apparent $K_m$ values of the reduced coenzyme substrates (Table III). NMN was an extremely poor inhibitor.

**Stereospecificity of NADPH Oxidation—**The stereospecificity of reduction by glycerol-P dehydrogenase was investigated by incubation of enzyme with saturating concentrations of dihydroxyacetone-P (1.0 mM) and either A-[4-3H]NADPH or B-[4-3H]NADPH (80 $\mu$m). The results for the wild type enzyme (Fig. 8) were identical with those for the feedback-resistant enzyme (data not shown). Approximately 90% NADPH was oxidized under these conditions. No oxidation occurred without enzyme. The separation of the reaction products in Solvent System I demonstrated the enzyme-dependent transfer of about 90% of the radioactivity from the B-labeled coenzyme to glycerol-P. About 90% of radiolabeled from the A-labeled coenzyme was associated with NADP. The results in Solvent System II demonstrated similar findings (data not shown). Therefore, wild type and feedback-resistant glycerol-P dehydrogenases have B-type stereospecificity.

**Discussion**

While the mechanisms which regulate the biosynthesis of membrane phospholipids in *E. coli* remain incompletely understood, significant progress has been made recently by combined biochemical, genetic, and physiological approaches (1, 19). Such investigations have established the biosynthetic role of the glycerol-P dehydrogenase (3, 4) and the physiological significance of the feedback inhibition by glycerol-P (6-8). Three considerations argued for in vivo regulation of glycerol-P synthesis: 1) the existence of the *plsB* phenotype (8); 2) the mass action ratios for the reaction catalyzed by the wild type for feedback-resistant glycerol-P dehydrogenases being far from equilibrium (8); and 3) the necessity of restricting the endogenous pool of glycerol-P to prevent the gratuitous induction of proteins and enzymes of the *gpl* regulon (21) which function in the catabolism of glycerol-P.

The present work on the characterization of the substrate specificity and on the specificity of inhibition of the wild type and feedback-resistant glycerol-P dehydrogenases was undertaken with an overall objective of providing additional evidence for a regulatory site for glycerol-P. The results will be discussed at the three levels of description, comparison, and interpretation of results to provide evidence for a glycerol-P regulatory site.

At the descriptive level, the glycerol-P dehydrogenase appeared to be moderately specific with respect to the ketose and aldehyde phosphates which it will utilize as substrates. Here, the most interesting finding was that the enzyme utilizes a 2-carbon aldehyde phosphate, glycolaldehyde-P (Table I). All of the substrates replacing dihydroxyacetone-P were poorer substrates having higher apparent $K_m$ values and lower maximal velocities (Table I). The two forms of glycerol-P dehydrogenase did not show any marked preference for NADPH, NADH, or nicotinamide hypoxanthine dinucleotide (Table III). The wild type and feedback-resistant glycerol-P dehydrogenase of *E. coli* had B-type stereospecificity for NADPH. The mammalian glycerol-P dehydrogenases studied also are B-type for NADH (22). This finding conforms to the generalization that this stereospecificity is highly conserved through evolution (22). It also conforms to the generalization that B-type stereospecificity is a property of oxidoreductases having phosphorylated substrates with more than 2 carbon atoms (22).

At the level of comparison of the wild type and feedback-resistant glycerol-P dehydrogenases, both had virtually identical kinetic constants for all of the sugar-phosphates (Table I) and reduced coenzyme substrates (Table III) tested. The inhibition of both enzymes by analogs of NADP were virtually identical (Table IV). These data provide further evidence that the active sites of the wild type and feedback-resistant glycerol-P dehydrogenases are virtually identical.

Additional evidence for the existence of a glycerol-P regulatory site is derived from studies employing the substrate-product pairs, 4-hydroxy-2-vinylbutyral 1-phosphonate/3,4-dihydroxybutyral 1-phosphonate and glycolaldehyde-P/ethylene glycol-P. The phosphonate analog of dihydroxyacetone-P proved to be a good substrate (Table I) and the phosphonate analog of glycerol-P was a potent competitive inhibitor (Table II). This substrate-product pair resembled that of dihydroxyacetone-P/glycerol-P in that the feedback-resistant enzyme was more than 10-fold less sensitive than the wild type enzyme (Table II). The mechanism of phosphonate reduction is sequential, like that of dihydroxyacetone-P (8), and since the Michaelis constant for NADPH was virtually identical for the dihydroxyacetone-P and its phosphonate analog, the kinetic mechanism appears to be ordered with NADPH binding to the free enzyme (8). The kinetic arguments for a glycerol-P regulatory site derived from the studies employing the phosphonate analogs are similar to those advanced previously and will not be repeated here (8). These studies are strongly contrasted by those employing the glycolaldehyde-P/ethylene glycol-P-substrate product pair. Ethylene glycol-P was a competitive inhibitor with respect to dihydroxyacetone-P, as was glycerol-P. The $K_m$ for glycolaldehyde-P as a substrate (Table I) was similar to the $K_m$ of ethylene glycol-P (Table II) for both wild type and feedback-resistant enzymes. The agreement between $K_m$ and $K_i$ values may be fortuitous. However, this result differed markedly from similar comparisons for dihydroxyacetone-P/glycerol-P or the phosphonate substrate-product pairs (Tables I and II) where the values differed by 32- and 40-fold for the wild type enzyme, respectively, and by 1.5- and 3.7-fold for the feedback-resistant enzyme, respec.
Biosynthetic Glycerol-P Dehydrogenase

It seems unlikely that ethylene glycol-P produces inhibition by the same mechanism as glycerol-P and the phosphonate analog. An interpretation of these findings is that glycerol-P and 3,4-dihydroxybutyl 1-phosphonate may inhibit through a regulatory site and ethylene glycol-P may inhibit through the active site.

The alternative substrate (glycolaldehyde-P) was a weak noncompetitive inhibitor with respect to dihydroxyacetone-P for the wild type and feedback-resistant enzymes. This non-competitive mode of inhibition may occur through the formation of E-NADPH·I and E-NADP·1 complexes, where I represents glycolaldehyde-P (14). Competitive inhibition would be the result, if only an E-NADPH·I complex were formed. The remaining compounds, which were neither substrates nor products of glycerol-P dehydrogenase, were weak competitive inhibitors with respect to dihydroxyacetone-P. All these compounds were equally potent inhibitors of the two enzymes except for P, which was a less potent inhibitor of the feedback-resistant enzyme. Thus, P, may act through the regulatory site. Since none of the physiological phosphorylated compounds tested produced potent inhibition of glycerol-P dehydrogenase in vivo, these compounds are unlikely to regulate glycerol-P synthesis in vivo.

Neither the present nor the previous kinetic investigations reveal significant differences in the kinetic properties of the wild type and feedback-resistant glycerol-P dehydrogenases in the absence of products. The reduced sensitivity of the feedback-resistant enzyme to inhibition by glycerol-P, 3,4-dihydroxybutyl 1-phosphonate, and P, appears to be the result of a mutation which spares the enzyme’s active site and involves a regulatory site for glycerol-P.

It is possible that a rapid equilibrium mechanism may operate for this substrate-product pair.

REFERENCES


SPECIAL TREATMENTS OF FERMENTATION CULTURES OF E. CLEAVA

A PROTOCOL FOR DEHYDROGENASE ACTIVITY  
CHARACTERIZATION OF WILD TYPE AND FEEDBACK-RESISTANT G. CLEAVA 3-PHOSPHOGLYCOLATE DEHYDROGENASE

J. Ralph Edgar and Robert M. Bell

TABLE I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wild Type</th>
<th>Feedback-Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycerol-P</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3,4-dihydroxybutyl 1-phosphate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>glycolaldehyde-P</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>not a substrate</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>Compound</th>
<th>Wild Type</th>
<th>Feedback-Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycerol-P</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3,4-dihydroxybutyl 1-phosphate</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>glycolaldehyde-P</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>not a substrate</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values were determined with various dihydroxyacetone-P and glutamine. All analyses were performed using glycerol-P and glycolaldehyde-P which were not subjected to

Values are based on a single concentration of substrate.
Biosynthetic Glycerol-P Dehydrogenase

Fig. 1. Kinetics of reduction of 3-hydroxy-2-methyl-3-phosphoglycerate by biosynthetic glycerol-P dehydrogenase. The kinetics of 3-hydroxy-2-methyl-3-phosphoglycerate reduction catalyzed by 34 μg of wild-type (Panel A) or feedback-resistant enzyme (Panel B) were determined and plotted as shown. The slopes of these plots were plotted against (mM NADH)² (Panel C and D).

Fig. 2. Kinetics of reduction of glycolaldehyde-P by biosynthetic glycerol-P dehydrogenase. The kinetics of glycolaldehyde-P reduction catalyzed by 34 μg of wild-type (Panel A) or feedback-resistant enzyme (Panel B) were determined and plotted as shown. The slopes of these plots were plotted against (mM NADH)² (Panel C and D).

Fig. 3. Inhibition of glycerol-P dehydrogenase by 3,4-dihydroxy-2-methyl-2-phosphoglycerate. The kinetics of glycerol-P dehydrogenase inhibition catalyzed by 34 μg of wild-type (Panel A) or feedback-resistant enzyme (Panel B) were determined and plotted as shown. The slopes of these plots were plotted against (mM NADH)² (Panel C and D).

Fig. 4. Inhibition of glycerol-P dehydrogenase by P-5,6-diphosphate. The kinetics of glycerol-P dehydrogenase inhibition catalyzed by 34 μg of wild-type (Panel A) or feedback-resistant enzyme (Panel B) were determined and plotted as shown. The slopes of these plots were plotted against (mM NADH)² (Panel C and D).