SUPPORTING INFORMATION

Phosphodianion Activation of Enzymes for Catalysis of Central Metabolic Reactions

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EXPERIMENTAL

Materials

The following reagent grade chemicals were obtained from Sigma-Aldrich; triethanolamine•HCl, NADH (disodium salt), NADP (disodium salt), D-xylose, D-xylonic acid (lithium salt), D-fructose 6-phosphate (sodium salt), D-glucose 6-phosphate (sodium salt). Bovine serum albumin (BSA) was obtained from Roche, and sodium phosphite (dibasic, pentahydrate) from Riedel de Haën was dried under vacuum at 25 °C for 16 hours before using. Water was from a Milli-Q Academic purification system. All other chemicals were reagent grade or better and were used without further purification.

6-Phosphogluconate dehydrogenase from *Escherichia coli* (*Ec*6PGDH) was purchased from Creative Biomart as crystals suspended in a saturated solution of ammonium sulfate. Glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (*Lm*G6PDH) was purchased as a lyophilized powder from Calzyme. Glucose 6-phosphate isomerase from *Saccharomyces cerevisiae* (*Sc*PGI, crystals

suspended in a saturated solution of ammonium sulfate) and sorbitol dehydrogenase from sheep liver (lyophilized powder) were purchased from Roche. The enzymes were prepared for use by extensive dialysis against 25 mM triethanolamine (TEA) buffer at pH 7.5 which, in the case of *Ec*6PGDH also contained 10 mM dithiothreitol (DTT). The protein concentrations for SDS gel electrophoresis were determined by the standard Bradford assay, with bovine serum albumin as calibration standard. The following molecular weights were calculated from the respective protein amino acid sequences: *Ec*6PGDH, 51481 Da;[1] *Lm*G6PDH Da, 54325;[2] *Sc*PGI, 61299 Da.[3] The concentration of stock solutions of *Ec*6PGDH, *Lm*G6PDH, and *Sc*PGI were determined from the UV absorbance at 280 nm and extinction coefficients of 59250, 69800, 73300 M^{-1} cm^{-1}, respectively, calculated using the ProtParam tool available on the ExPASy server.[4-5]

The purity of the commercial enzymes used in this work was examined by SDS gel electrophoresis. The enzymes were exhaustively dialyzed against water and then diluted to a concentration of 0.2 mg/mL. Each enzyme was mixed with an equal volumes of 2X Laemmli buffer (Bio-Rad) supplemented with β-mercaptoethanol (5% v/v). The protein solutions were heat-denatured at 80 °C for 15 min, and 10 µL (1 µg) of each protein was loaded into wells of a 12% SDS-PAGE gel. The proteins were separated at 200 V in 25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS, pH 8.3. The final gel was stained with Coomasie Blue; and, a digital image was obtained using a desktop scanner.

Preparation of Solutions. The solution pH was determined as described previously.[6] Stock solutions of NADP and NADH were prepared by dissolving the solid cofactors (sodium salts) in water. The concentration of NADP in stock solutions was

determined spectrophotometrically from the change in absorbance at 340 nm upon quantitative enzyme-catalyzed reduction to form NADPH using an extinction coefficient of 6220 M^{-1} cm^{-1}. Stock solutions of sodium phosphite were prepared by dissolving the solid salt in water. The concentration of phosphite dianion in solutions at pH 7.5 was calculated from the total sodium phosphite concentration using a pK_a of 6.38 for conversion of phosphite monoanion to the dianion.[6]

Stock solutions of 6-phosphogluconate, glucose 6-phosphate, and fructose 6-phosphate were prepared by dissolving the solid sugar phosphates in water. The concentration of 6-phosphogluconate and glucose 6-phosphate in these stock solutions was determined, respectively, as the concentration of NADPH formed upon quantitative oxidative decarboxylation to ribulose 5-phosphate catalyzed by *Ec*6PGDH and quantitative oxidation to 6-phosphogluconate lactone catalyzed by *Lm*G6PDH. The concentration of fructose 6-phosphate in stock solutions was determined as the concentration of NADPH formed upon quantitative *Sc*PGI-catalyzed isomerization to glucose 6-phosphate followed by *Lm*G6PDH-catalyzed oxidation of glucose 6-phosphate to 6-phosphogluconate lactone.

Stock solutions of D-xylonate and D-xylose were prepared by dissolving the solid sugars in water. A large gravimetric preparation D-xylose stock solution was made to minimize uncertainty. This was done by dissolving 950 mg of the analytical reagent (purity > 0.99) in water (Milli-Q filtration system) in a volumetric flask (25.00 ± 0.03 mL). Aliquots of this 0.250 M stock solution were frozen at –20 °C and thawed on the day of use. The concentration of D-xylonate was determined independently, using ^{1}H-NMR at 500 MHz in D_2O, from the relative areas of the peaks for the methylene protons

of D-xylonate and for an acetone internal standard. There was good agreement (±1%) between the concentrations of D-xylonate determined by weight and by ^{1}H-NMR.

Enzyme Assays. All enzyme assays were in a volume of 1.0 mL. The reactions were monitored using either a temperature controlled Cary 3500 Multicell Peltier UV-Vis spectrophotometer or a Cary 3E spectrophotometer equipped with a temperature-controlled Peltier block multicell changer. The initial velocities for reactions of the whole phosphorylated substrates were determined by monitoring < 10% of substrate reaction, and the initial velocities for reaction of the phosphodianion truncated substrates were determined by monitoring < 1% of substrate reaction.

Enzyme Catalyzed Reactions of Whole Substrates. The oxidative decarboxylation of 6-phosphogluconate to form ribulose 5-phosphate catalyzed by *Ec*6PGDH,[7] and the oxidation of glucose 6-phosphate to form the lactone of 6-phosphogluconate catalyzed by *Lm*G6PDH[8] were followed by monitoring the increase in absorbance at 340 nm from formation of the reduced product NADPH. The isomerization of fructose 6-phosphate to form glucose 6-phosphate catalyzed by *Sc*PGI was followed by coupling formation of glucose 6-phosphate to the reduction of NADP catalyzed by *Lm*G6PDH, and monitoring the increase in absorbance at 340 nm.[9]

These reactions were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.125, NaCl), 0.1 mg/mL BSA, 0.02 mM EDTA (*Lm*G6PDH only), 1.0 mM NADP for *Ec*6PGDH and *Lm*G6PDH and 0.45 mM NADP for *Sc*PGI, and the appropriate substrate. The reactions were initiated by addition of enzyme to give the following final concentrations: 3 nM *Ec*6PGDH, 0.27 nM *Lm*G6PDH and 0.05 nM

*Sc*PGI, with 4 units of the *Lm*G6PDH coupling enzyme. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for 10–30 min.

Enzyme-Catalyzed Reactions of Phosphodianion-Truncated Substrates. *Ec6PGDH.* The unactivated reactions of D-xylonate catalyzed by *Ec*6PGDH were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.14, NaCl), 20 mM NADP, and 3–24 mM D-xylonate. The reactions were initiated by addition of *Ec*6PGDH to give a final enzyme concentration of 3 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for 12 h.

The phosphite dianion-activated reactions of D-xylonate catalyzed by *Ec*6PGDH were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.14, NaCl), 20 mM NADP, 3–24 mM D-xylonate, and up to 30 mM sodium phosphite. The reactions were initiated by addition of *Ec*6PGDH to give final enzyme concentrations of 1–4 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for 30 min.

LmG6PDH. The unactivated reactions of D-xylose catalyzed by *Lm*G6PDH were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.125, NaCl), 0.1 mg/mL BSA, 0.02 mM EDTA, 1 mM NADP, and 10–50 mM D-xylose. The reactions were initiated by addition of *Lm*G6PDH to give a final enzyme concentration of 1.1 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for 12 h.

The phosphite dianion-activated reactions of D-xylose catalyzed by *Lm*G6PDH were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.125, NaCl), 0.1 mg/mL BSA, 0.02 mM EDTA, 1 mM NADP, 10–50 mM D-xylose, and 5–25 mM

sodium phosphite. The reactions were initiated by addition of *Lm*G6PDH to give a final enzyme concentration of 0.27 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for 30 min.

ScPGI. The unactivated reactions of D-xylose catalyzed by *Sc*PGI were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.125, NaCl), 0.1 mg/mL BSA, 0.36 mM NADH, 10–50 mM D-xylose, and 0.3 units of sorbitol dehydrogenase. The reactions were initiated by addition of *Sc*PGI to give a final enzyme concentration of 3.0 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for a period of 12 h.

The phosphite dianion-activated reactions of D-xylose catalyzed by *Sc*PGI were at pH 7.5 and 25 °C in solutions that contain 25 mM TEA buffer (I = 0.125, NaCl), 0.1 mg/mL BSA, 0.36 mM NADH, 10–50 mM D-xylose, 5–25 mM sodium phosphite, and 0.3 units of sorbitol dehydrogenase. The reactions were initiated by addition of *Sc*PGI to give final enzyme concentrations of 1.5–3.0 μM. The initial reaction velocity was determined by monitoring the increase in absorbance at 340 nm for a period of 12 h.

The initial reaction velocities were calculated from the change in absorbance at 340 nm using an extinction coefficient of 6200 M^{-1} cm^{-1} for NADH and NADPH. The nonlinear least squares fits of plots of kinetic data reported in the main manuscript to the appropriate kinetic equations were obtained using Prism 8 for MacOS from GraphPad Software.

RESULTS

Figure S1 shows a digital scan of the Coomasie Blue-stained gel obtained from SDS-PAGE gel electrophoresis of 1 μg of each of the following commercial enzymes: *Escherichia coli* 6-phosphogluconate dehydrogenase (*Ec*6PGDH), *Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase (*Lm*G6PDH), *Saccharomyces cerevisiae* glucose 6-phosphate isomerase (*Sc*PGI), and sheep liver sorbitol dehydrogenase (SDH). Only a single major band was observed for each of these four proteins.

Figures S1A, S1B, and S1C show plots of v/[E] against [substrate], where v is the initial velocity for the enzyme-catalyzed reactions of 6-phosphogluconate, glucose 6-phosphate, and fructose 6-phosphate, respectively, catalyzed by *Ec*6PGDH, *Lm*G6PDH, and *Sc*PGI. The following kinetic parameters were determined from the fit of these kinetic data to the Michaelis–Menten equation: *Ec*6PGDH-catalyzed reaction of 6-phosphogluconate: k_{cat} = 12.0 ± 0.1 s^{-1}, K_m = 14.3 ± 0.7 μM; *Lm*G6PDH-catalyzed oxidation of glucose 6-phosphate: k_{cat} = 320 ± 10 s^{-1}, K_m = 160 ± 10 μM; *Sc*PGI-catalyzed isomerization of fructose 6-phosphate to glucose 6-phosphate: k_{cat} = 398 ± 2 s^{-1}, K_m = 168 ± 3 μM. The values for the second-order rate constant, $(k_{cat}/K_m)_{SPi,}$ determined for these enzyme-catalyzed reactions of phosphorylated substrates are reported in Table 1 of the main manuscript.

Figures S3A, S3B, and S3C show plots of v/[E] against [substrate], where v is the initial reaction velocity for the enzyme-catalyzed reactions of truncated substrates D-xylonate, D-xylose, and D-xylose, respectively, catalyzed by *Ec*6PGDH, *Lm*G6PDH, and *Sc*PGI. The values for the second-order rate constant, $(k_{cat}/K_m)_S$, determined for the

enzyme-catalyzed reactions of phosphodianion-truncated substrates are determined as the slopes of the linear correlations from Figures S3A and S3B. The following kinetic parameters were determined from the fit of kinetic data for Figure S3C: K_m = 80 ± 10 mM and $(k_{cat}/K_m)_S$ = (3.6 ± 0.2) x10^{-4} M^{-1} s^{-1}.

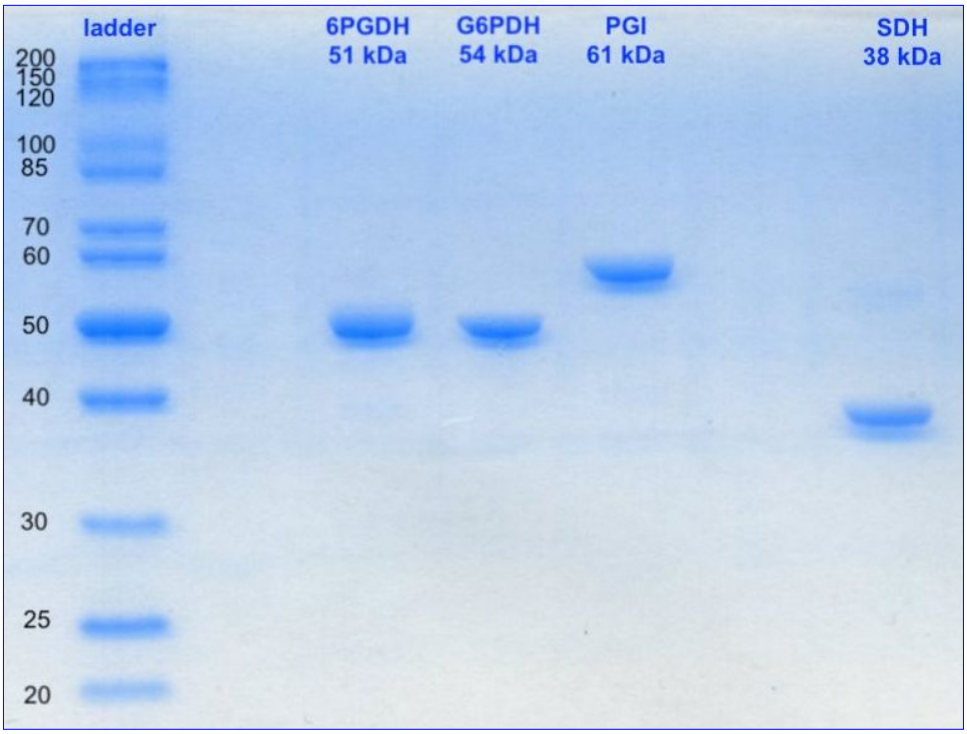

Figure S1. SDS-PAGE gel of 1 µg of each of the following enzymes: *Escherichia coli* 6-phosphogluconate dehydrogenase, *Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase, *Saccharomyces cerevisiae* glucose 6-phosphate isomerase, and *sheep* liver sorbitol dehydrogenase (SDH).

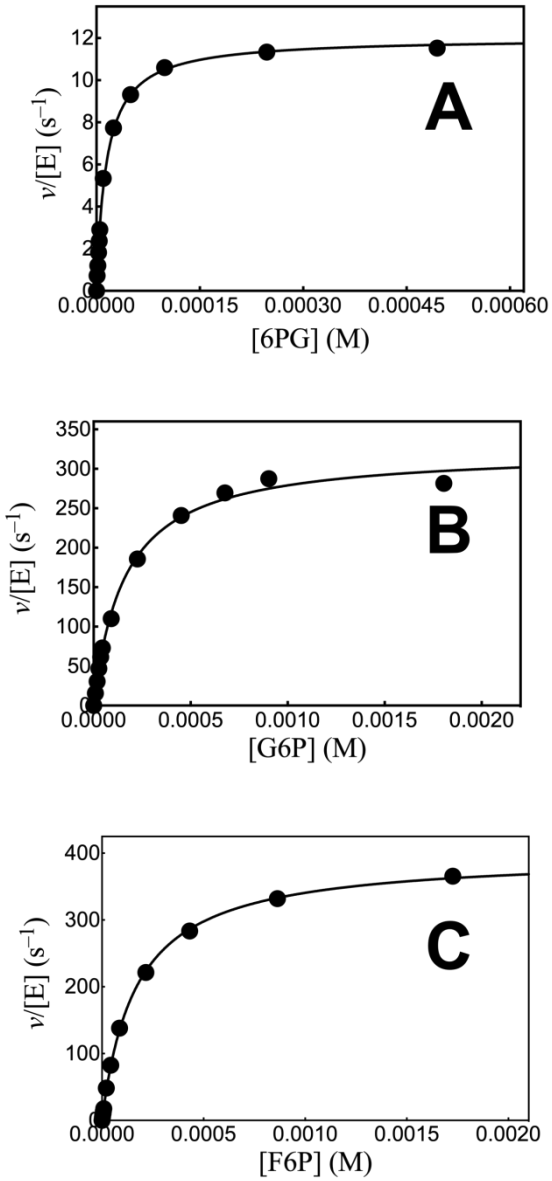

Figure S1. Michaelis–Menten plots for reactions catalyzed by *Ec*6PGDH, *Lm*G6PDH, and *Sc*PGI at pH 7.5 (TEA buffer) and 25 °C. (A) *Ec*6PGDH-catalyzed oxidative decarboxylation of 6-phosphogluconate. (B) *Lm*G6PDH-catalyzed oxidation of glucose 6-phosphate by NADP. (C) *Sc*PGI-catalyzed isomerization of fructose 6-phosphate.

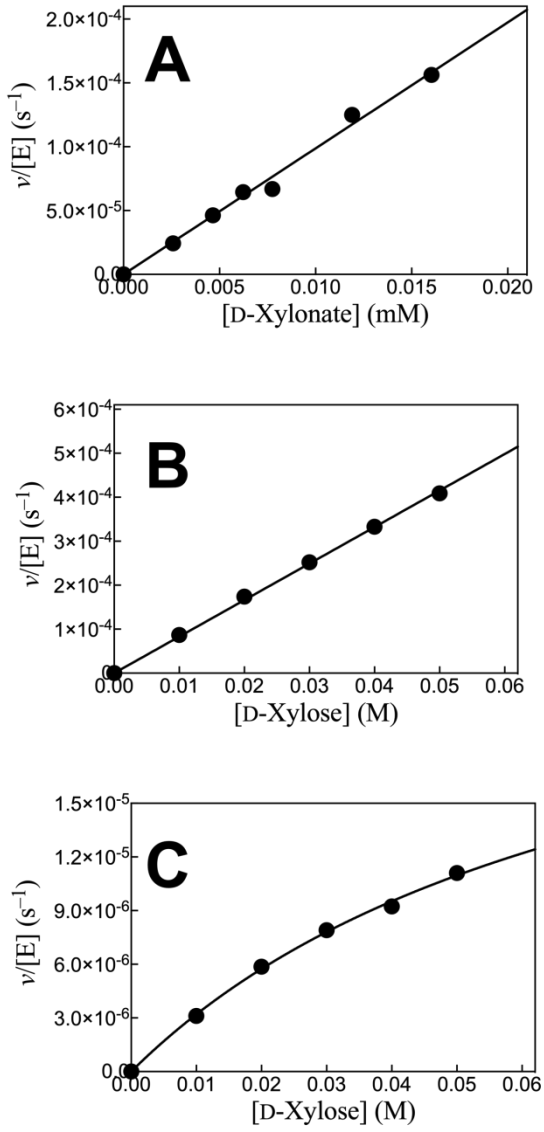

Figure S3. Plots of *v*/[E] against the concentration of D-xylonate or D-xylose for reactions catalyzed by *Ec*6PGDH, *Lm*G6PDH, and *Sc*PGI at pH 7.5 (TEA buffer) and 25 °C. (A) *Ec*6PGDH-catalyzed oxidation of D-xylonate. (B) *Lm*G6PDH-catalyzed oxidation of D-xylose by NADP. (C) *Sc*PGI-catalyzed isomerization of D-xylose.

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