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Selective Inhibition of Metabolic Enzymes by Enzymatically Synthesized D-Glucal-6-Phosphate§

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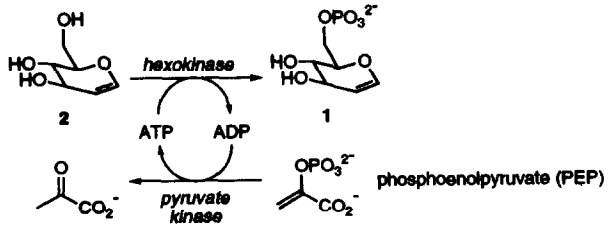
Abstract—Yeast hexokinase (EC 2.7.1.1) catalyzes the phosphorylation of D-glucal and methyl α- and β-D-glucopyranosides at 1–5 % of the rates of phosphorylation of D-glucose and 2-deoxy-D-glucose. Maltose, cellobiose, D-galactal and tetrahydropyran-2-methanol are not substrates of hexokinase. Enzymatically synthesized D-glucal-6-phosphate inhibits rabbit muscle phosphoglucose isomerase competitively (K_I = 1.94 mM) and phosphoglucomutase noncompetitively (K_I = 0.122 mM).

Sugar phosphates are important intermediates in mammalian and microbial metabolism. Selective inhibitors of enzymes that act upon sugar phosphates may be useful as tools for biomedical research or as therapeutic agents. As part of a program aimed at the preparation and study of the physical and biological properties of sugar phosphates, we have examined the ability of yeast hexokinase (EC 2.7.1.1) to catalyze the phosphorylation of derivatives and analogs of glucose. Unnatural substrates have been phosphorylated on preparative (1 g) scale. D-Glucal-6-phosphate (**1**), which was prepared in one step (Scheme I) by enzymatic phosphorylation of D-glucal (**2**), inhibits some but not all enzymes that act upon glucose-6-phosphate (G6P) as their substrate. Enzymes that are inhibited by **1** differ both in the magnitude (K_I) and in the pattern (competitive or noncompetitive) of their inhibition. We note that noncompetitive inhibitors are particularly attractive as potential therapeutic agents since their effects on the velocity of an enzyme-catalyzed reaction are independent of the concentration of substrate present.[1]

In vivo, hexokinase catalyzes the phosphorylation of the C-6 hydroxyl group of α- and β-glucopyranose.[2,3] ATP is the phosphoryl donor. We have found that hexokinase also catalyzes the phosphorylation of methyl α- and β-D-glucopyranosides and D-glucal (Table 1). 2-Deoxy-D-glucose is as good a substrate as glucose itself. Tetrahydropyran-2-methanol (**3**), however, is not phosphorylated by hexokinase. Apparently, at least one or two secondary hydroxyl groups must be present for a compound to be a substrate of hexokinase, even though no single secondary hydroxyl group of glucose is essential for substrate activity. Analogs of glucose lacking any one of its secondary hydroxyl groups are known to be substrates of hexokinase.[4,5] D-Galactal (**4**) and the 1,4-linked dimers of glucose, maltose and cellobiose, are not substrates of hexokinase. The inactivity of **4** is not surprising since D-galactose is not phosphorylated by hexokinase.[6]



Scheme I.

Table 1. Relative rates of phosphorylation of potential substrates catalyzed by yeast hexokinase

compound	relative activity
D-glucose	100
2-deoxy-D-glucose	96
methyl α-D-glucopyranoside	5.0
methyl β-D-glucopyranoside	0.3
D-glucal (**2**)	0.2
tetrahydropyran-2-methanol (**3**)	< 0.05
maltose	< 0.05
cellobiose	< 0.05
D-galactal (**4**)	< 0.03

Potential substrates were 50 mM in 0.2 M sodium phosphate buffer, pH 7.5.

The conversion of **2** to **1** served as a useful test of the preparative capabilities of hexokinase-catalyzed phosphorylations. Because of the cost of the cofactor, only a catalytic quantity of ATP was used as the immediate phosphoryl donor, and ATP was regenerated *in situ* using pyruvate kinase and phosphoenolpyruvate (PEP) as the ultimate phosphoryl donor (Scheme I).[7,8] When enzymatic assay of pyruvate in the reaction indicated 88 % conversion

§This paper is dedicated to Professor J. Bryan Jones on the occasion of his sixtieth birthday.

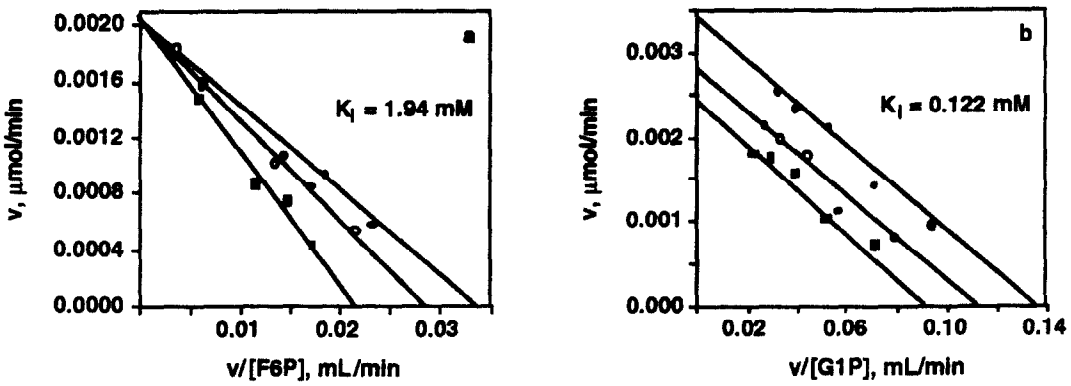
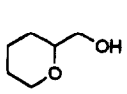
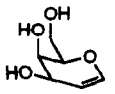


Figure 1. Inhibition of metabolic enzymes by D-glucal-6-phosphate (**1**). (a) Rabbit muscle phosphoglucose isomerase. Concentrations of **1** were 0.00 mM (●), 0.33 mM (○), and 1.00 mM (■). Lines were calculated using $V_{max} = 2.05 \times 10^{-3}$ µmol min^{-1}, $K_m = 6.12 \times 10^{-2}$ mM, and K_I = 1.94 mM. (b) Rabbit muscle phosphoglucomutase. Concentrations of **1** were 0.000 mM (●), 0.025 mM (○), and 0.050 mM (■). Lines were calculated using $V_{max} = 3.40 \times 10^{-3}$ µmol min^{-1}, $K_m = 2.53 \times 10^{-2}$ mM, and $K_I = 0.122$ mM.

of **2**, the solution (62 mM product) was adjusted to pH 9, diluted with one volume of water and one volume of methanol, and applied to a column of Dowex® 1 × 8–200 (bicarbonate) anion exchange resin. The column was rinsed, eluted with 65 mM ammonium bicarbonate in 50 % aqueous ethanol to remove pyruvate, and then eluted with 220 mM ammonium bicarbonate to give **1** (diammonium salt) in 99 % yield, based on the extent of conversion. The crude product contained ammonium phosphate as its only impurity. Purification of **1** as its bis(cyclohexylammonium) salt gave 1.1 g (94 % yield, based on extent of conversion).[9] Although **2** reacted with hexokinase at only 0.2 % of the rate of the natural substrate, the synthesis of **1** required only $5.85 worth of enzyme. Immobilization and reuse of the enzyme could lower the effective cost of the catalyst even further.



tetrahydropyran-2-methanol (**3**)



D-galactal (**4**)

Because **1** is a nonmetabolizable analog of G6P, we investigated its ability to inhibit enzymes that act upon G6P as their natural substrate. We expected the flattening of the ring caused by the double bond in **1** to influence the ability of **1** to bind to various enzymes. We detected no inhibition of hexokinase (glucose as substrate) by 1 mM **1**, and we observed no product inhibition during the synthesis of **1**. Similarly, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was essentially unaffected by **1**. Compound **1** is only a weak competitive inhibitor, with K_I being nearly two orders of magnitude greater than K_m for G6P.

Compound **1** does inhibit phosphoglucose isomerase (EC 5.3.1.9) isolated from rabbit muscle (K_I = 1.94 mM, Figure 1) or yeast (K_I = 2.28 mM, data not shown). The inhibition is competitive, and the values of K_I are about 25–30 times the values of K_m for fructose-6-phosphate (F6P). Although we envision **1** as an antagonist of G6P, the kinetics of phosphoglucose isomerase were measured with F6P as the substrate since the rates are operationally simpler to determine in the direction forming G6P rather than in the direction consuming G6P. Since both F6P and G6P bind to the same active site of the enzyme, we expected **1** to be competitive with respect to either substrate.

The inhibition of rabbit muscle phosphoglucomutase (EC 5.4.2.2) by **1** is noncompetitive (Figure 1) with respect to α-glucopyranose-1-phosphate (G1P), despite the fact that G1P and G6P presumably bind to the same active site of the same phosphorylated form of the enzyme.[10] The value of K_I (0.122 mM) is only about five times the value of K_m for G6P.

Our results indicate that a single substrate analog can have significantly different inhibitory effects on enzymes that share a common substrate. Although the four enzymes studied share G6P as their substrate, they differ both in the magnitudes and patterns of their inhibition by **1**. The potential for noncompetitive inhibition, as in the case of phosphoglucomutase, is notable since the effects of noncompetitive inhibition cannot be attenuated by high concentrations of substrate.[1]

Acknowledgments

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9. ^{1}H NMR (250 MHz, D_2O) δ 6.42 (dd, J = 1.6, 6.1 Hz, 1H, H-1), 4.81 (dd, J = 2, 6.2 Hz, 1H, H-2), 4.28 (dt, J = 1.9, 7.3 Hz, 1H, H-3), 4.15 (ddd, J = 3.4, 8.2, 12.2 Hz, 1H, H-6), 4.00 (ddd, J = 1.7, 6.1, 12.4 Hz, 1H, H-6'), 3.90 (br d, J = 11 Hz, 1H, H-5), 3.82 (dd, J = 7.2, 10.0 Hz, 1H, H-4). ^{31}P NMR (D_2O, 85 % H_3PO_4 ext. ref.) δ 5.77.

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