Short Communication

In Vitro Inhibition of the Plastid and Cytosolic Isozymess of 6-Phosphogluconate Dehydrogenase from Developing Endosperm of Ricinus communis by Fructose 2,6-bisphosphate

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ABSTRACT

Activities of the cytosolic and plastid isozymes of 6-phosphogluconate dehydrogenase from developing endosperm of Ricinus communis L. seeds were inhibited in vitro by hexosebisphosphates. Inhibition constants for glucose 1,6-bisphosphate were 221 and 209 micromolar for the cytosolic and plastid isozymes, respectively, and corresponding values for fructose 2,6-bisphosphate were 10.5 and 8.6 micromolar. In each case inhibition was of a mixed noncompetitive nature relative to 6-phosphogluconate. While the levels and distribution of fructose 2,6-bisphosphate in castor oil seed endosperm cells are not yet known, the levels reported to occur in leaf cytosol would be high enough to significantly inhibit carbon flux through the pentose phosphate pathway due to inhibition of 6-phosphogluconate dehydrogenase activity.

The role of F2,6-P2 in regulation of glucose homeostasis in mammalian liver cells (7) and in yeast (2, 9) has been extensively examined. It is becoming increasingly more evident that F2,6-P2 is also a potent regulator of carbohydrate metabolism in plants. It has been demonstrated that F2,6-P2 stimulates glycolysis at the level of F6P conversion to F1,6-P2 in several plant tissues (5, 8, 10, 12, 13) and inhibits gluconeogenesis by inhibiting FBPase activity (5, 17). A recent report suggests that F2,6-P2 may also regulate sugar nucleotide metabolism in plants (6).

While it has been firmly established that F2,6-P2 has the potential to regulate glycolysis/gluconeogenesis in plant cells, there have as yet been no reports concerning a possible role for this effector in modulation of pentose-phosphate cycle activity. Blackmore and Shuman (4) have, however, suggested that F2,6-P2 can alter the flux through the pentose-phosphate pathway in perfused rat liver. It was previously reported that G1,6-P2 inhibited the in vitro activity of both isozymes of 6-PGDH from cultured tobacco cells. As G1,6-P2 serves as a less active analog of F2,6-P2 in stimulating the in vitro activity of plant PPI: fructose-6-phosphate 1-phosphotransferase (12, 13), we undertook an examination of the in vitro effect of F2,6-P2 on the isozymes of 6-PGDH (14) from the developing endosperm of Ricinus communis.

MATERIALS AND METHODS

Castor oil plants, Ricinus communis L., cv Baker 296, were glasshouse grown and homogenates of developing endosperm prepared as previously described (11). The isozymes of 6-phosphogluconate dehydrogenase were separated and purified to homogeneity by a modification (manuscript in preparation) of the method of Simcox and Dennis (15). The activity of 6-PGDH was measured as described by Simcox and Dennis (15). Enzyme assays were conducted at room temperature (approximately 23°C) using a Cary 210 spectrophotometer and were linear with respect to time and amount of added enzyme.

Chromatographic matrices for enzyme purification were from Pharmacia and Bio-Rad. Biochemicals, including G1,6-P2 and F2,6-P2, were from the Sigma Chemical Company. NADP was from Boehringer Mannheim.

RESULTS AND DISCUSSION

As with 6-PGDH from various rat tissues (3) and tobacco cells (1), the enzyme from developing Ricinus endosperm was inhibited by G1,6-P2 (Table I). In Table I values for the rat enzymes were from 20 to 40 μM. At saturating concentrations of 6-PG, the 6-PGDH isozymes from tobacco cells were only slightly inhibited by G1,6-P2, but at ¼ km (approximately 20 μM) the I0.5 were

<table>
<thead>
<tr>
<th>6-PGDH Isozyme</th>
<th>G1,6-P2</th>
<th>F2,6-P2</th>
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</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>221</td>
<td>10.5</td>
</tr>
<tr>
<td>Plastid</td>
<td>209</td>
<td>8.6</td>
</tr>
</tbody>
</table>

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3 Abbreviations: F2,6-P2, fructose 2,6-bisphosphate; 6-PGDH, 6-phosphogluconate: NADP+ oxidoreductase [decarboxylating] (EC 1.1.1.44); G1,6-P2, glucose 1,6-bisphosphate; F6P, fructose-6-phosphate; F1,6-P2, fructose 1,6-bisphosphate; FBPase, fructose 1,6-bisphosphate 1-phosphohydrolase (EC 3.1.3.11).
approximately 250 μM for both isozymes (1). The $K_i$ values for the 6-PGDH isozymes from developing Ricinus endosperm were approximately 200 μM (Table I), assayed at 0.3 mM 6-PG. Under identical conditions F2,6-P$_2$ was a much more powerful inhibitor of in vitro 6-PGDH activity (Table I) with $K_i$ values of approximately 10 μM. Lower $K_i$ values were estimated when assays were conducted at 6-PG concentrations equal to or less than Km values, however, nonlinearity of the assays rendered these measurements unreliable. The inhibition appeared to be a mixed noncompetitive nature with $V_{max}$ much more effected than $K_m$ (Fig. 1).

We have previously reported that F2,6-P$_2$ has the potential to stimulate both plastid (10) and cytosolic (12) glycolysis in the developing endosperm of Ricinus seeds. While FBPases from photosynthetic tissues are inhibited by F2,6-P$_2$, developing castor oil seeds lack significant FBPase activity in either the plastids or cytosol. This is perhaps not surprising as gluconeogenesis is not a significant activity of this tissue (16). As with animal tissues and yeast, stimulation of glycolysis and inhibition of gluconeogenesis in plants is accomplished by nm concentrations of F2,6-P$_2$ (5, 8, 10, 12, 13, 17). In view of the profound in vitro effects induced by nm concentrations of F2,6-P$_2$, it might seem that inhibition of 6-PGDH activity by 10 μM F2,6-P$_2$ represents only an interesting aside. It has, however, been reported that leaves may contain F2,6-P$_2$ at concentrations approaching 5 μM, while Cseke et al. (5) suggest that concentrations of up to 300 μM may be present. If a recent report that cellular F2,6-P$_2$ is confined to the cytosol is correct, then concentrations of F2,6-P$_2$ in this compartment could be greater than initially observed. F2,6-P$_2$ concentrations of this magnitude significantly inhibit in vitro 6-PGDH activity and may also be of in vivo importance. Inhibition of 6-PGDH activity in situ would serve to increase the carbon flux through glycolysis, compatible with the concomitant stimulation of the conversion of F6P to F1,6-P$_2$. 

**Fig. 1.** Inhibition of the cytosolic isozyme of Ricinus 6-PGDH by fructose 2,6-P$_2$. Panel A, Assay conditions as described in Table I. Assays were conducted in the presence of 0, 1, 5, or 10 μM F2,6-P$_2$. Panel B, Replot of the intercepts as a function of inhibitor concentration.

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