Communication

Effects of Fructose 2,6-Bisphosphate on Phosphoglucomutase from Plants

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ABSTRACT

Fructose 2,6-bisphosphate affects phosphoglucomutase from plant and animal sources in a similar way. As previously found with rabbit muscle phosphoglucomutase, fructose 2,6-bisphosphate cannot substitute for glucose 1,6-bisphosphate as a cofactor in the reaction catalyzed by phosphoglucomutase from potato tubers, pea seeds, and string-beans. In the presence of glucose 1,6-bisphosphate, fructose 2,6-bisphosphate inhibits phosphoglucomutase from potato tubers. Activation of phosphoglucomutase from plant sources by fructose 2,6-bisphosphate reported by others was probably due to contamination of the commercial preparation of fructose 2,6-bisphosphate by glucose 1,6-bisphosphate.

MATERIALS AND METHODS

Chemicals. Glucose 1-P, glucose 6-P, glu-1,6-P$_2$, fructose 6-P, fructose 1,6-P$_2$, glycerate 3-P, glycerate 2,3-P$_2$, NADP, ATP, glucose 6-P dehydrogenase from yeast, and phosphoglucomutase from rabbit muscle were obtained from Boehringer. Fru-2,6-P$_2$ was a kind gift from Dr. E. Van Schaftingen (Brussels) or purchased from Sigma. Glucose 1-P was freed of glu-1,6-P$_2$ by ion-exchange chromatography (12). DE-Cellulose P-23 was from Whatman and Sephadex G-150 was from Pharmacia. All the other reagents were analytical grade.

Plant Material. Pea seeds (Pisum sativum L.), string-beans (Phaseolus vulgaris L.) and potatoes (Solanum tuberosum L.) were obtained from a local market.

Extraction of Tissues. All steps were carried out at 2 to 4°C. Plant tissues were homogenized with a Sorvall Omnimixer homogenizer (3000-5000 rpm, 2-3 min) in 2 vol (v/w) of 100 mM Tris-HCl buffer (pH 7.5) containing 10 mM EDTA and 10 mM β-mercaptoethanol. After centrifugation at 2000g for 30 min, the filtered cheesecloth supernatants were submitted to fractionated precipitation with poly(ethylene glycol) 8000 (5-15%). The pellets were resuspended in a minimum volume of extraction buffer.

Partial Purification of Potato Tuber Phosphoglucomutase. Since potato extract contains enzymic activities (phosphoglucomutase and phosphofructokinase) which could interfere with phosphoglucomutase assay, potato tuber phosphoglucomutase was partially purified as follows. All steps were carried out at 2 to 4°C. The extract (10 ml, 15-20 mg protein/ml) was applied to a DE-Cellulose P-23 column (6 × 1.5 cm) equilibrated with homogenization buffer. The column was washed with equilibrating buffer until the $A_{280}$ reached the original value, and was eluted, at flow rate of 40 ml/h, first with 50 ml of equilibrating buffer supplemented with 50 mM KCl and then with 50 ml of equilibrating buffer containing 200 mM KCl. Phosphoglucomutase activity emerged with the flow-through fractions. The pooled activity (7-8 ml, 4-6 mg/ml) was applied to a Sephadex G-150 column (145 × 1.5 cm) and eluted with homogenization buffer at a flow rate of 5 ml/h. Phosphoglucomutase emerged as a single peak which was found to be free of interfering enzymes.

Enzyme Assay. Phosphoglucomutase was assayed using glucose 6-P dehydrogenase in a coupled reaction (11). The assay mixture contained in a total volume of 1 ml, in a 1-cm light-path cell equilibrated at 30°C: 500 μM glucose 1-P, 500 μM NADP, 10 mM MgCl$_2$, 32 mM histidine, 40 mM Tris-HCl buffer (pH 7.5), 0.3 unit of glucose 6-P dehydrogenase, and the sample to be tested. After 10 min preincubation, 500 nm glut-1,6-P$_2$ was added, and the increase in the $A_{340}$ was followed. In contrast with mammalian phosphoglucomutase, the enzyme from plants is

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2 Abbreviations: glu-1,6-P$_2$, glucose 1,6-bisphosphate; fru-2,6-P$_2$, fructose 2,6-bisphosphate; glycerate 2,3-P$_2$, glycerate 2,3-bisphosphate.
Table I. Effect of Glu-1,6-P2 and Fru-2,6-P2 on Phosphoglucomutase from Plants

<table>
<thead>
<tr>
<th>Enzyme Source</th>
<th>G-1,6-P2</th>
<th>F-2,6-P2 (Sigma)</th>
<th>F-2,6-P2 (E.V.S.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 50</td>
<td>5 50</td>
<td>5 50</td>
</tr>
<tr>
<td>Potato tubers*</td>
<td>87 128</td>
<td>5.8 29</td>
<td>ND* ND</td>
</tr>
<tr>
<td>Pea seedsb</td>
<td>18.7 21</td>
<td>1.6 8.2</td>
<td>ND* ND</td>
</tr>
<tr>
<td>String beansb</td>
<td>10 12</td>
<td>1.5 6</td>
<td>ND* ND</td>
</tr>
</tbody>
</table>

* Partially purified enzyme.  b Tissue extract.  c Not detectable.

Table II. Inhibition of Potato Tuber Phosphoglucomutase by Bisphosphorylated Compounds

<table>
<thead>
<tr>
<th>Addition</th>
<th>Concentration of Phosphorylated Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 μM</td>
</tr>
<tr>
<td>% of the control value</td>
<td>100*</td>
</tr>
<tr>
<td>None</td>
<td>80</td>
</tr>
<tr>
<td>Fructose 2,6-P2 (E.V.S.)</td>
<td>74</td>
</tr>
<tr>
<td>Fructose 1,6-P2</td>
<td>74</td>
</tr>
<tr>
<td>Glycerate 2,3-P2</td>
<td>73</td>
</tr>
</tbody>
</table>

* Control value, 128 milliunits/mg protein.

The assay. Phosphoglucoisomer and phosphofructokinase were assayed as described by Van Schaftingen et al. (14).

Measurement of Metabolites and Protein. Glu-1,6-P2 was determined spectrophotometrically as cofactor of rabbit muscle phosphoglucomutase (2). Fru-2,6-P2 was assayed as activator of potato pyrophosphate:fructose 6-P phosphotransferase (14). Protein was determined by the method of Mokrash and McGilvery (8), using BSA as a standard.

RESULTS AND DISCUSSION

As summarized in Table I, phosphoglucomutase activity of extracts from potato tubers, pea seeds, and string beans was activated by glu-1,6-P2 and by fru-2,6-P2 from Sigma, but not by fru-2,6-P2 from Dr. E. Van Schaftingen.

Figure 1 shows the effects of the bisphosphorylated sugars on phosphoglucomutase partially purified from potato tubers. The enzyme showed no activity in the absence of added glu-1,6-P2, the K<sub>s</sub> being calculated to be 1.9 μM. Fru-2,6-P2 from Sigma produced some activating effect. The effect persisted when the commercial preparation was incubated under acid conditions (0.7 N HCl at 30°C for 30 min) where fru-2,6-P2 is hydrolyzed. It was abolished after heating under conditions (0.7 N HCl at 90°C for 60 min) where glu-1,6-P2 is hydrolyzed. On basis of the amount of the glu-1,6-P2 calculated to be present as contaminant in fru-2,6-P2 from Sigma (1–2%), a 20% increase in phosphoglucomutase activity would be expected after fru-2,6-P2 hydrolysis, due to the release from fru-2,6-P2 inhibition (see below). Such increase is reduced by the partial hydrolysis of glu-1,6-P2 (20%) produced under the conditions that destroy fru-2,6-P2, and by the inhibition of phosphoglucomutase activity (10%) by the salts present in the hydrolyzed and neutralized solution. In contrast with fru-2,6-P2, Sigma, fru-2,6-P2 from Dr. E. Van Schaftingen (E. V. S.), which does not activate muscle phosphoglucomutase (2), did not stimulate potato tuber phosphoglucomutase.

As shown in Table II, in the presence of glu-1,6-P2, phosphoglucomutase partially purified from potato tubers is inhibited by fru-2,6-P2. In the presence of 500 μM glucose 1-P and 500 nM glu-1,6-P2, the inhibitory effect produced by fru-2,6-P2 is slightly less than that produced by fructose 1,6-P2 and glyceraldehyde 2,3-P2. Both fructose 1,6-P2 and glyceraldehyde 2,3-P2 have been shown to inhibit phosphoglucomutase from rabbit muscle (2, 7) and from beef liver (4). No inhibition was observed when glu-1,6-P2 and fru-2,6-P2 were present at the same concentration (100 μM).

The results herein reported show that fru-2,6-P2 affects phosphoglucomutase from plant and from animal sources in a similar way. As previously found with rabbit muscle phosphoglucomutase (2), fru-2,6-P2 cannot substitute for glu-1,6-P2 as a cofactor in the interconversion of glucose 1-P and glucose 6-P catalyzed by phosphoglucomutase from potato and other plants. Furthermore, in the presence of glu-1,6-P2, fru-2,6-P2 inhibits phosphoglucomutase from potato tubers. The activation of plant phosphoglucomutase by fru-2,6-P2 (Sigma) reported by others (5) was

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probably an artifact due to the presence of glu-1,6-P_2 in the commercial preparation (2). The fact that activation of phosphoglucomutase from plant sources by glu-1,6-P_2 and by fru-2,6-P_2 reached a different plateau was used as an argument against the possibility that activation by fru-2,6-P_2 was due to contamination with glu-1,6-P_2. However, the inhibitory effect of fru-2,6-P_2 on phosphoglucomutase activity in the presence of glu-1,6-P_2 explains the lower activatory effect produced by the mixture of bisphosphorylated sugars present in the commercial preparation of fru-2,6-P_2.

Recently (3), glu-1,6-P_2 has been reported to be present in plants. The physiological meaning of phosphoglucomutase inhibition by fru-2,6-P_2 would depend on the relative proportion of glu-1,6-P_2 and fru-2,6-P_2 in plant tissues.

LITERATURE CITED