Pyrophosphate: fructose 6-phosphate phosphotransferase in germinating castor bean seedlings

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The distribution of enzymes interconverting fructose 6-phosphate and fructose 1,6-bisphosphate has been studied in a range of tissues from castor bean seedlings. In each tissue the activity of PP_i: fructose 6-phosphate phosphotransferase was greater than phosphofructokinase and substantial compared with fructose 1,6-bisphosphatase. PP_i: fructose 6-phosphate phosphotransferase in endosperm is apparently confined to the cytoplasm. The role of this latter enzyme in vivo is discussed.

Castor bean *PP_i: fructose 6-phosphate phosphotransferase* *Phosphofructokinase* *Fructose 1,6-bisphosphatase* *Glycolysis* *Gluconeogenesis*

1. INTRODUCTION

Pyrophosphate: fructose 6-phosphate 1-phosphotransferase has been measured in several plant tissues [1–4]. The enzyme in mung bean hypocotyl [2], spinach leaf [3], and potato tuber [4] is strongly stimulated by Fru-2,6-P_2, a potent activator of some mammalian phosphofructokinases [5]. Our knowledge of the role of PFP in plant metabolism is limited. No direct comparison has been made between this enzyme and PFK and there is little evidence to suggest that PFP is quantitatively significant in any of the above tissues. The activity extracted from spinach leaves [3] was 2–3-times greater than estimates for the activity of PFK from the same tissue [6], but neither report provides evidence that maximum enzyme activities were measured, hence the comparison is inconclusive. Here, we demonstrate that PFP is present throughout castor bean seedlings at activities comparable to those of both PFK and FBPase, the two enzymes of Fru-6-P/Fru-1,6-P_2 metabolism generally believed to be important in glycolysis and gluconeogenesis, respectively. Fractionation studies with endosperm extracts show that PFP is not associated with any major organelle.

Abbreviations: PFP, PP_i: fructose 6-phosphate phosphotransferase (EC 2.7.1.90); PFK, phosphofructokinase (EC 2.7.1.11); FBPase, fructose 1,6-bisphosphatase (EC 3.1.3.11); Fru-6-P, fructose 6-phosphate; Fru-1,6-P_2, fructose 1,6-bisphosphate; Fru-2,6-P_2, fructose 2,6-bisphosphate.

2. MATERIALS AND METHODS

Castor bean seeds (*Ricinus communis* var. Hale) were soaked for 24 h in running tap water, then placed in moist vermiculite (time zero) and grown in the dark at 30°C in a humidified growth chamber. To obtain green cotyledons and leaves, seeds were grown for 20 days in a 12 h light: 12 h dark regime at 30°C and watered with Hoagland's solution.

For measurement of maximum catalytic activities, the appropriate tissue from 5 seedlings was homogenized in 4–20 vol. 100 mM triethanolamine–HCl buffer (pH 7.7), 1 mM $MgCl_2$, 1 mM EDTA, 14 mM 2-mercaptoethanol. Examination of the homogenates with a microscope revealed very few unbroken cells. The homogenate was centrifuged at 30 000 × *g* for 20 min and 1 ml portions of the supernatant passed through a column (1 × 7 cm) of Bio-Gel P-2 equilibrated with extrac-

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tion buffer. The de-salted extract was used for the enzyme determinations.

In the localization experiments endosperms from 30 4-day-old plants were chopped with razor blades for 15–20 min in 15 ml medium containing 150 mM Tricine–KOH (pH 7.5), 0.4 M sucrose, 5 mM EDTA, 0.1% (w/v) bovine serum albumin. The resulting brei was filtered through 2 layers of nylon mesh and the filtrate was centrifuged at 20000 × *g* for 15 min. The pellet was resuspended in 5–7 ml of the extraction medium.

All enzyme assays were carried out at 25°C in 1 ml total vol. unless specified otherwise. Fru-2,6-P_2 was purchased from Sigma. Coupling enzymes, obtained from Sigma and Boehringer, were dialysed before use. NADH oxidation and $NADP^+$ reduction were recorded at 340 nm with a Gilford 250 spectrophotometer. The reaction mixture for PFP activity contained 100 mM Hepes–NaOH buffer (pH 7.3), 1 mM $MgCl_2$, 10 mM Fru-6-P, 0.1 mM NADH, 0.2 mM PP_i, 1 μM Fru-2,6-P_2, 1 U aldolase, 10 U triosephosphate isomerase, 1 U glycerol-3-phosphate dehydrogenase and 1 U hexose phosphate isomerase. The reaction was started by the addition of PP_i. Assay conditions for PFK were identical to those for PFP, except that 5 mM $MgCl_2$ was present and 1 mM ATP replaced PP_i, while no Fru-2,6-P_2 was added. For FBPase the reaction mixture contained 100 mM Hepes–NaOH buffer (pH 7.3), 5 mM $MgCl_2$, 1 mM $NADP^+$, 0.5 mM Fru-1,6-P_2, 1 U hexose phosphate isomerase, 1 U glucose 6-phosphate dehydrogenase and 0.25 U 6-phosphogluconate dehydrogenase. The reaction was started by the addition of Fru-1,6-P_2. Other enzymes were assayed: catalase [7], fumarase [8], ribulose 1,5-bisphosphate carboxylase (in 0.5 ml) [9]; phospho*enol*pyruvate carboxylase (in 0.5 ml) [10]; NADH-cytochrome *c* reductase (antimycin A-resistant) [11]. Protein was measured according to [12] with bovine serum albumin as a standard.

3. RESULTS

Estimates of the activities of PFP, PFK and FBPase in various tissues from castor bean seedlings are presented in table 1. The addition of Fru-2,6-P_2 to the assay stimulated PFP by 2–10-fold, but had no measurable effect on PFK. We confirmed that the assays gave optimum activity by varying the concentration of each component, and the pH, of the reaction mixture for each tissue. To check whether our measurements were affected by enzyme inhibitors or activators in any of the tissues we prepared a series of extracts each containing two different tissues. The activity of each enzyme measured in these mixtures was 84–124% of those measured in the tissues extracted separately. These results demonstrate that the measurements in table 1 represent the maximum catalytic activities of the various tissues.

We studied the endosperm and cotyledons in more detail. The activities of all 3 enzymes were measured in both tissues during the week following germination. In the endosperm (fig. 1) the enzymes display a pattern of development similar to many of the enzymes involved in gluconeogenesis [13]. In cotyledons PFP, PFK and FBPase all in-

Table 1

Enzyme activities in tissues of castor bean seedlings

Tissue	Fresh weight (g.seedling^{-1})	Protein (mg.g fresh wt^{-1})	Activity (μmol.min^{-1}.g fresh wt^{-1})		
			PFP	PFK	FBPase
Endosperm	0.763 ± 0.048	26.68 ± 2.82	0.659 ± 0.030	0.222 ± 0.018	0.951 ± 0.025
Cotyledons	0.049 ± 0.003	105.42 ± 3.21	6.516 ± 0.290	1.444 ± 0.148	1.477 ± 0.182
Hypocotyl	0.147 ± 0.021	26.97 ± 2.65	2.476 ± 0.246	0.240 ± 0.037	0.352 ± 0.044
Roots	0.356 ± 0.027	9.17 ± 0.79	0.536 ± 0.094	0.084 ± 0.015	0.104 ± 0.013
Green cotyledons	0.478 ± 0.119	22.50 ± 2.42	0.281 ± 0.020	0.184 ± 0.007	0.398 ± 0.025
Leaves	0.461 ± 0.050	32.29 ± 1.21	0.583 ± 0.093	0.249 ± 0.020	0.633 ± 0.033

Enzyme activity was measured in de-salted extracts of tissues of 4-day-old seedlings, except for green cotyledons and leaves which were from plants grown in the light for 20 days. For leaves the fresh weight refers to a pair of leaves containing ~2.2 mg chlorophyll.g fresh wt^{-1}. Values represent the mean ± SE of 4 separate expt.

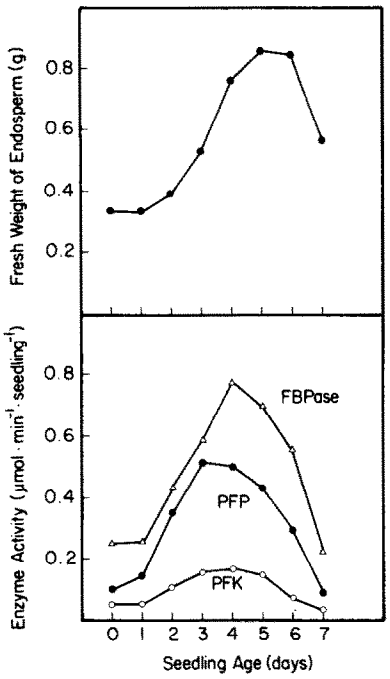

Fig. 1. Enzyme activities in the endosperm during seedling development. Each value represents the mean of measurements from 2 separately grown batches of plants.

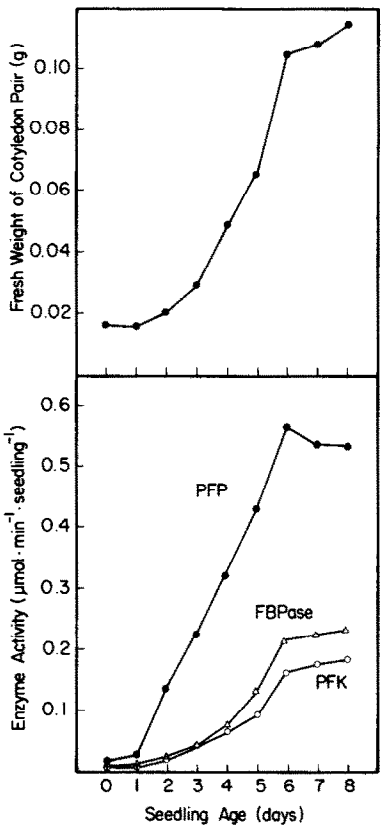

Fig. 2. Enzyme activities in the cotyledons during seedling development. Each value represents the mean of measurements from 2 separately grown batches of plants.

crease steadily during early growth with PFP exhibiting the largest increase in activity (fig. 2).

To determine the subcellular location of PFP in endosperm we measured the distribution of activity in the pellet and supernatant after centrifugation of an endosperm extract (table 2). The pellet contained major proportions of plastids, mitochondria, glyoxysomes and endoplasmic reticulum as indicated by the distribution of RuBP carboxy-

Table 2

Subcellular localization of PFP in castor bean endosperm

Enzyme	Activity in unfractionated homogenate (nmol.min^{-1}.mg protein^{-1})	Percentage of activity in unfractionated homogenate recovered in:	
		20 000 × *g* pellet	pellet + supernatant
PFP	17.89 ± 0.84	3.03 ± 0.26	106.5 ± 2.5
PEP carboxylase	6.95 ± 0.43	3.17 ± 0.41	98.9 ± 4.7
RuBP carboxylase	7.19 ± 0.56	51.0 ± 7.9	87.0 ± 6.0
Fumarase	241.1 ± 16.5	91.5 ± 4.0	105.0 ± 5.2
Catalase	101.9 ± 6.9	80.9 ± 1.8	100.4 ± 3.4
NADH-cytochrome *c* reductase	53.27 ± 1.8	46.4 ± 1.5	86.8 ± 1.0

Extracts prepared by chopping 4-day-old endosperms with razor blades were centrifuged at 20 000 × *g* for 15 min. Enzyme activities were measured in the unfractionated extract, pellet and supernatant. Catalase activity was calculated according to [7] and is given as units.mg protein^{-1}. All values represent the mean ± SE of 3 separate expt.

lase, fumarase, catalase and NADH-cytochrome *c* reductase, respectively. Contamination of the pellet by cytoplasm or whole cells was slight as indicated by the distribution of PEP carboxylase [14]. The distribution of PFP was very similar to that of PEP carboxylase showing that this too is located in the cytoplasm. We emphasize that for each enzyme the sum of activity in the pellet plus supernatant was similar to that in the unfractionated homogenate (table 2). Thus the distribution of enzymes was not distorted by enzyme activation or inactivation during fractionation.

4. DISCUSSION

The results demonstrate that PFP has the capacity to be quantitatively important in sugar phosphate metabolism in castor bean. PFP was present at all stages in all the tissues investigated and in each the activity was 2–10-times greater than that of PFK. PFP activity also exceeded FBPase activity in the cotyledons, hypocotyl and roots.

The similarity of PFP and FBPase activities in the endosperm contrasts markedly with the excess of PFP in the other non-photosynthetic tissues. Since in the endosperm the flux is in the direction of sucrose synthesis, whereas in these other tissues glycolysis predominates, the distribution of enzymes might suggest that PFP functions in the glycolytic direction in vivo. Based on the kinetic properties of the enzyme from potato tubers it was proposed that PFP works in the direction of Fru-6-P consumption [4].

However, the reaction catalysed by PFP is easily reversible in vitro ($\Delta G^{\circ\prime} = -2.93$ kJ.mol^{-1}) and the possibility that this enzyme plays a major role in sucrose synthesis in castor bean endosperm by contributing to Fru-6-P production cannot be dismissed. All the PFP is apparently located in the cytoplasm, the site of gluconeogenesis [14]. From estimates of the maximum in vivo rate of sucrose synthesis [15] the flux from Fru-1,6-P_2 to Fru-6-P is ~0.30 μmol.min^{-1}.g fresh wt^{-1}. The activity of PFP is more than sufficient to account for this rate. In a subsequent publication we will report on the kinetic properties of PFP from castor bean endosperm which may help to understand the role of the enzyme in vivo.

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