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Comparison of the Activities and Some Properties of Pyrophosphate and ATP Dependent Fructose-6-Phosphate 1-Phosphotransferases of *Phaseolus vulgaris* Seeds[1]

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ABSTRACT

The distribution of pyrophosphate: fructose 6-phosphate phosphotransferase (PFP) and ATP: fructose-6-phosphate 1-phosphotransferase (PFK) was studied in germinating bean (*Phaseolus vulgaris* cv Top Crop) seeds. In the cotyledons the PFP activity was comparable with that of PFK. However, in the plumule and radicle plus hypocotyl, PFP activity exceeds that of PFK. Approximately 70 to 90%, depending on the stage of germination, of the total PFP and PFK activities were present in the cotyledons. Highest specific activity of both enzymes, however, occurred in the radicle plus hypocotyl (64–90 nanomoles · min · milligram protein). Fractionation studies indicate that 40% of the total PFK activity was associated with the plastids while PFP is apparently confined to the cytoplasm. The cytosolic isozyme of PFK exhibits hyperbolic kinetics with respect to fructose 6-P and ATP with K_m values of 320 and 46 micromolar, respectively. PFP also exhibits hyperbolic kinetics both in the presence and absence of the activator fructose-2,6-P_2. The activation is caused by lowering the K_m for fructose 6-P from 18 to 1.1 millimolar and that for pyrophosphate (PPi) from 40 to 25 micromolar, respectively. Levels of fructose 2,6-P_2 and PPi in the seeds are sufficient to activate PFP and thereby enable a glycolytic role for PFP during germination. However, the fructose 6-P content appears to be well below the K_m of PFP for this compound and would therefore preferentially bind to the catalytic site of PFK, which has a lower K_m for fructose 6-P. The ATP content appears to be at saturating levels for PFK.

The reaction catalyzed by PFK[2] (EC 2.7.1.11) is widely accepted as the most probable site of regulation of glycolytic flux in higher plants. The activity of PFK has been shown to be regulated by a variety of effectors including adenine nucleotides, P-enolpyruvate, citrate, and Pi (for review, see Ref. 32). The discovery of pyrophosphate:fructose 6-phosphate 1-phosphotransferase (PFP, EC 2.7.1.90) in a wide range of plants (5–7, 16, 18, 25, 34) led to the suggestion that the conversion of fru-6-P to fru-1,6-P_2 involves an additional enzyme. In plants PFP appears to be a cytosolic enzyme (6, 7, 18) that reversibly interconverts fru-6-P/fru-1,6-P_2 and PPi/Pi. Fructose-2,6-P_2 is a powerful activator of PFP at nanomolar concentrations (7, 14, 25, 29, 34, 36). Depending on the degree of activation by fru-2,6-P_2, PFP appears to be also regulated by several effectors including glycerate 3-P, citrate, adenine nucleotides, and Pi (15).

During germination of several species an increase in glycolysis occurs (21, 22, 30, 31, 33). In *Phaseolus mungo* (19) and *Phaseolus vulgaris* (26) the production of alcohol and lactic acid during germination also strongly suggest an active glycolytic flux.

Present knowledge regarding the role of PFP in seed metabolism during germinating is limited. Only in castor bean (18), black gram seeds (2), and peas (36) have changes in PFP activity during germination been reported. Only in two cases were PFP and PFK activity compared (2, 18). However, in only one of these reports was evidence provided that maximum activities were measured (18). In plant tissue PFK activity is present in both the cytosol and plastids (for review, See Ref. 8). Competition between PFP and PFK for the common substrate fru-6-P will therefore be largely restricted to cytosolic glycolysis.

Here we demonstrate that PFP is present at activities higher than or comparable to PFK activity in different parts of *P. vulgaris* seeds during germination. In addition, it is shown that PFK is present in both the cytosol and plastids, while PFP is not associated with any major organelle. We also measured PPi and fru-2,6-P_2 to establish whether these compounds were present at sufficient levels to permit PFP to act in the glycolytic direction.

MATERIALS AND METHODS

Materials. *Phaseolus vulgaris* L. cv Top Crop seed was purchased from a local seed supplier and stored in airtight glass containers at 20°C in the dark. PEG-8000, fru-2,6-bisphosphate and sodium pyrophosphate were from Sigma while enzymes, with the exception of PFP, other substrates and cofactors were from Boehringer-Mannheim Biochemicals. Chromatography matrices and PD-10 columns were obtained from Pharmacia Fine Chemicals. Picozyme F was obtained from Packard Instrument Co. Panacide (5,5′-2,2′-dihydroxydiphenylmethane) was obtained from British Drug House Chemicals. Solvents and other reagents employed were of analytical grade. PFP was purified from potato tubers (35).

Methods. *Germination and Isolation of Seed Parts.* The seeds were surface sterilized in 0.5% (m/v) Panacide for 5 min at room temperature and then thoroughly rinsed with running distilled H_2O. Germination tests were conducted in 500 ml Schott Duran glass bottles capped with airtight lids. Seeds were incubated at 27°C in the dark in these bottles on a single layer of Schleicher and Schüll No. 595 filter paper moistened with 8 ml distilled H_2O. To avoid the development of anaerobic conditions the bottles were opened every 12 h for 10 min. A seed was considered

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[2] Abbreviations: PFK, ATP:fructose 6-phosphate phosphotransferase (EC 2.1.7.11); PFP, PPi:fructose 6-phosphate phosphotransferase (EC 2.1.7.90); fru-6-P, fructose 6-phosphate; fru-2,6-P_2, fructose 2,6-bisphosphate; fru-1,6-P_2, fructose 1,6-bisphosphate; RuBP, ribulose 1,5-bisphosphate; TPI, triose phosphate isomerase (EC 5.3.1.1); FBPase, fructose 1,6-bisphosphatase (EC 3.1.3.11); ADH, alcohol dehydrogenase (EC 1.1.1.1).

germinated when the radicle had emerged.

At various incubation times, the testae were removed and the cotyledons separated from the axes. Axes were further divided into plumules and radicles plus hypocotyls.

Enzyme Extraction and Purification. For the measurement of maximum catalytic activities, the appropriate tissue from 8 seeds was homogenized with an ultroturrax for 1 min in 5 ml (plumules), 10 ml (radicles plus hypocotyl), or 20 ml (cotyledons, whole seeds) 100 mM Hepes buffer (pH 7.5) containing 1 mM $MgCl_2$, 1 mM EDTA, and 14 mM 2-mercaptoethanol. The homogenate was centrifuged at 27 000*g* for 15 min and the supernatant used in the enzyme assays. Aliquots of the extracts were desalted by passing through a PD-10 column.

For the partial purification of PFK and PFP, 20 g of seeds (72 h of incubation) were used. After extraction in 40 ml buffer and centrifugation as detailed above, PEG 8,000 was added to the supernatant (5 g·100 ml^{-1}) with continuous stirring. The preparation was allowed to stand for 10 min and then centrifuged at 10 000*g* for 10 min. A further 9 g·100 ml^{-1} PEG was then added to the resulting supernatant, mixed and after 30 min, centrifuged as described above. The resulting pellet was dissolved in 10 mM Hepes buffer (pH 7.5) containing 1 mM $MgCl_2$ and 14 mM 2-mercaptoethanol and applied to a DEAE-Sephacel column (2.5 × 10 cm) equilibrated with the same buffer. The column was washed with 60 ml of buffer and developed with a gradient of KCl (0–500 mM) in the same buffer. The flow rate was 60 ml·h^{-1} and 4 ml fractions were collected. The most active fractions of the PFK activity eluting at a conductivity of 14 mmho·cm^{-1} were pooled, mixed with 1 volume cold glycerol, and used within 4 h. The most active PFP fractions were pooled, and then concentrated by adding solid $(NH_4)_2SO_4$ to 60% saturation; after centrifugation at 15,000*g* for 15 min, the precipitate was dissolved in 10 mM Mes (pH 6.6) containing 10 mM 2-mercaptoethanol, 1 mM $MgCl_2$, and 10% glycerol and applied to a Sephacryl S300 column (1.5 × 90 cm) equilibrated in the same buffer. The column was washed in the same buffer and the fractions containing most of the PFP activity were pooled. The extract was then applied to a phosphocellulose column (2.5 × 10 cm) equilibrated in the same buffer followed by a linear gradient of 0 to 500 mM KCl in equilibration buffer. The fractions containing most of the PFP activity were pooled and dialyzed overnight in 10 mM Hepes-NaOH buffer (pH 7.5) containing 30% glycerol, 7 mM 2-mercaptoethanol, and then stored at −20°C.

In the localization experiments 20 seeds were taken 72 h after the onset of incubation and homogenized with a chilled mortar and pestle in 1.5 volumes of 100 mM Tris buffer (pH 7.5) containing 2 mM EDTA, 10 mM 2-mercaptoethanol, and 0.5 M mannitol. After filtering through two layers of nylon cloth the filtrate was centrifuged at 500*g* for 5 min. The pellet was discarded and the supernatant layered on a 6 ml sucrose cushion containing 10 mM Tris buffer (pH 7.5) and 12% (w/w) sucrose and centrifuged at 27,000*g* for 30 min. The pellet was resuspended in 5 ml extraction buffer.

Enzyme Assays. All assays were performed at 25°C and activities were linear with respect to both time and enzyme concentration. Absorbance was measured with a Cary 219 spectrophotometer. Coupling enzymes were dialyzed against 10 mM Hepes-NaOH (pH 7.5) before use. Acid stable radioactivity was determined with a LKB RackBeta liquid scintillation counter, and readings were corrected for quenching using the external standard method.

Unless otherwise indicated, the reaction mixture contained the following components in a final volume of 1 ml. For PFP activity the standard reaction mixture contained 100 mM Hepes (pH 8.0), 1 mM $MgCl_2$, 10 mM fru-6-P, 0.15 mM NADH, 1 mM $Na_2H_2P_2O_7$, 10 units TPI, 1 unit glycerol 3-P dehydrogenase, 1 unit fru-1,6-P_2 aldolase, and 10 μM fru-2,6-P_2. The reaction mixture for PFK was the same except that the pH was 7.5, fru-2,6-P_2 was omitted, and 1 mM ATP replaced PPi. To confirm that the assays gave maximum activity the concentration of each component and the pH of the reaction mixture were varied for each tissue. The presence of possible enzyme activators and inhibitors was determined by preparing a series of extracts each containing at least two different tissues. The measured activity in these extracts was 94 to 104% of that measured in the tissues extracted separately.

Inorganic pyrophosphatase was assayed in a mixture containing 100 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, and 5 mM PPi. The reaction was stopped after 10 min of incubation by adding TCA to a final concentration of 5% (w/v). After centrifugation at 13,000*g* for 4 min the Pi in the supernatant was estimated (13). The following enzymes were assayed as described previously: RuBP carboxylase (EC 4.1.1.39) (10), alcohol dehydrogenase (EC 1.1.1.1) (23), fumarase (EC 4.2.1.2) (24), and FBPase (EC 3.1.3.11) (18).

Measurement and Extraction of Metabolites. At the appropriate incubation times metabolites were extracted from the seeds. Two seeds were used for each extract. For the extraction of ATP, fru-6-P, and PPi the seeds were freeze clamped, dropped into liquid N_2 pulverized, extracted in 2 ml 1.41 M $HClO_4$, and neutralized as described in (9). Fructose-2,6-P_2 was extracted from the freeze clamped tissue in a methanol/chloroform mixture (29). Fru-6-P, PPi, and fru-2,6-P_2 were assayed using purified potato PFP. For the measurement of fru-6-P and PPi the assay mixture contained in 1 ml:50 mM Hepes, 2 mM magnesium acetate, 0.15 mM NADH, 10 μM fru-2,6-P_2, 1 unit aldolase, 1 unit glycerol 3-P dehydrogenase, 10 units TPI, and up to 0.8 ml neutralized extract. For the measurement of fru-6-P the assay mixture also contained 0.5 mM PPi and for measurement of PPi it contained 5 mM fru-6-P. The reaction was started by addition of 0.1 unit PFP and allowed to go to completion. Fru-2,6-P_2 was assayed by its activation of PFP from potato tubers (34). The amount of fru-2,6-P_2 in each sample was calculated by comparing the extent of activation with that induced by known amounts of authentic fru-2,6-P_2. No activation was found in extracts incubated in 0.1 M HCl for 15 min at 25°C. ATP was measured using the luciferin-luciferase method. The assay mixture contained in a volume of 0.25 ml:80 mM Tris (pH 7.5), 2 mM $MgSO_4$, and 0.04 ml Picozyme F (reconstituted in 2 ml distilled H_2O). Luminescence of the assay mixture was determined for 30 s, after a preincubation of 5 s following the addition of Picozyme F, on a LKB 1251 luminometer. Readings were corrected for quenching by the internal standard method. Amount of ATP was calculated using an ATP calibration curve, 10^{-10} to 10^{-6} M ATP.

Kinetic Analysis. Kinetic constants were determined at the pH optimum. Kinetic data were analyzed using a nonlinear regression program (11).

Other Analytical Methods. Conductivity was measured with a Philips PW 9526 conductivity meter. Protein was measured by the method of Bradford (4) using bovine γ-globulin as a standard.

RESULTS

In preliminary tests the germination of *Phaseolus vulgaris* seed at 27 ± 0.5°C in the dark was investigated. The first radicles protruding through the testae were visible in the period from 16 to 24 h of incubation and 90 ± 4% germination was attained after 96 h of incubation (Fig. 1).

Total PFP activity increased during the first 48 h of incubation then declined up to 96 h of incubation (Fig. 1). The total PFK activity increased during the first 16 h of incubation and then remained constant up to 72 h whereafter it declined. The PFP activity measured in the presence of 5 μM fru-2,6-P_2 was higher than the total PFK activity throughout the 96 h incubation period (Fig. 1). However, in the absence of fru-2,6-P_2, PFK

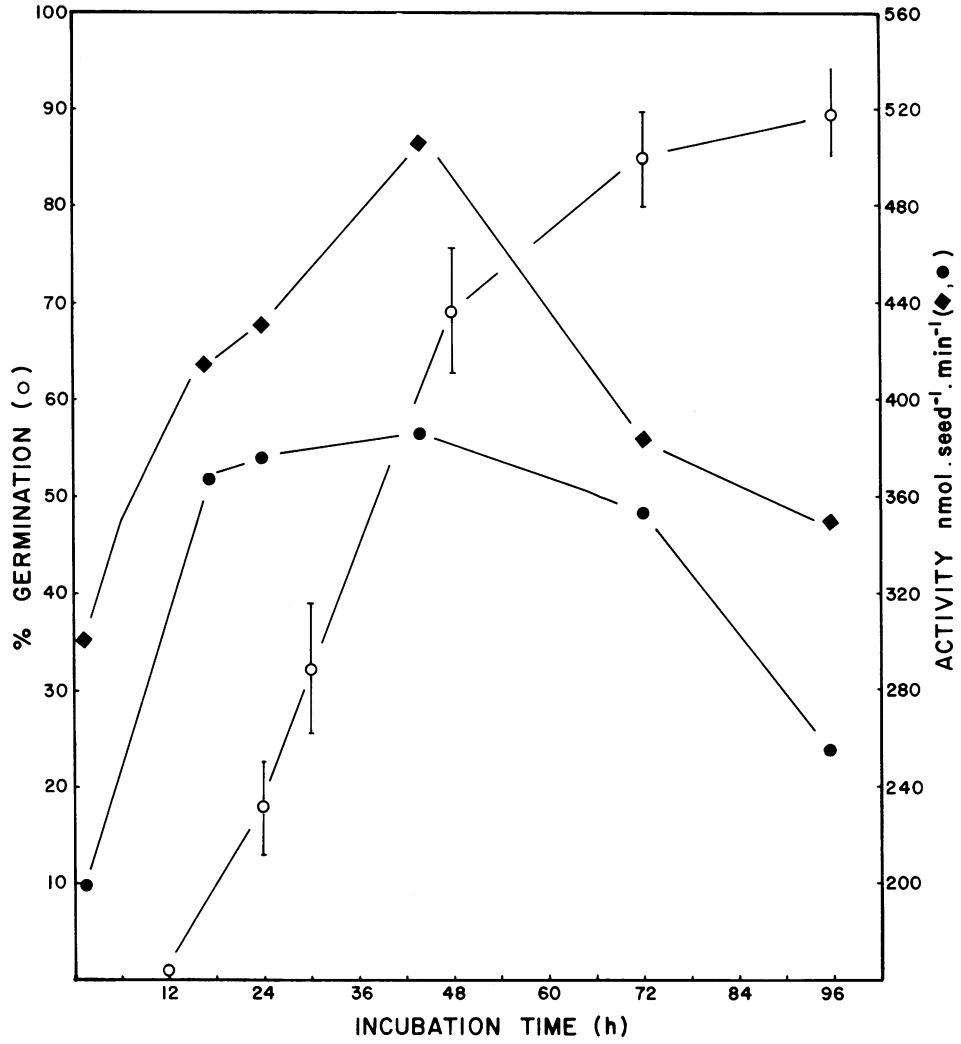

FIG. 1. Changes in total PFP activity (◆) and PFP activity (●) during germination (○) of bean seeds at 27°C in the dark.

activity was approximately 9 times higher than PFP activity.

The addition of fru-2,6-P_2 stimulated PFP activity by 7- to 24-fold (Fig. 2), but had no measurable effect on PFK. Highest and lowest activation of PFP activity was found in the dry seeds and after 72 to 96 h of incubation, respectively.

The subcellular localization of PFP and PFK was determined by measuring the distribution of activity in the supernatant and pellet after centrifugation (Table I). The pellet contained large portions of mitochondria and plastids as indicated by the distribution of RuBP carboxylase and fumarase activity. Contamination of the pellet by cytoplasm and whole cells was slight as indicated by the distribution of ADH activity. The distribution of PFP was similar to that of ADH, suggesting that PFP is located in the cytoplasm. In contrast, a portion of the PFK activity is associated with the pellet. Assuming that this PFK activity was associated with the plastids, and correcting for plastid yield using the distribution of RuBP carboxylase as an indicator, it was calculated that up to 38% of the total PFK is associated with the plastids. The total PFK activity was also separated into two fractions on a DEAE-Sephacel column, eluting at a conductivity of 14 and 21 mmho·cm^{-1}, respectively. Chromatography of a plastid enriched fraction confirmed that the peak at a conductivity of 21 mS·cm^{-1} was PFK activity associated with the plastids (results not shown). The ratio between the two PFK peaks also confirmed a distribution of approximately 3:2 between the cytosol and plastid PFK activities.

The cytosolic PFK isozyme (PFK_c) was unstable after the DEAE-Sephacel chromatography step. Approximately 80% activity was lost over a 24 h period. For this reason PFK_c was used in kinetic studies without any further purification. The specific activity of the PFK_c preparation was 350 nmol·min^{-1}·mg^{-1} protein, while the specific activity of the partially purified PFP enzyme was 6.5 μmol·min^{-1}·mg^{-1} protein. The final PFP preparation was free from aldolase and PFK activity while pyrophosphatase and FBPase activities were less than 0.05 and 0.5% of the PFP activity, respectively. Both PFP and PFK have a pH optimum between 7.2 and 7.8 and exhibit normal hyperbolic kinetics with fru-6-P/PPi and fru-6-P/ATP, respectively (results not shown). The kinetic constants of PFK and PFP are shown in Table II. Fru-2,6-P_2 has no effect on PFK but PFP activity is greatly stimulated (Table II). The activation of PFP is caused by a decrease in the K_m of the enzyme for substrate, especially fru-6-P, as well as an increase in V_{max}.

Estimates of the levels of fru-6-P, fru-2,6-P_2, ATP, and PPi at different stages of germination are presented in Table III. Highest ATP levels were found at 24 and 48 h of incubation followed by a decrease. The PPi level between 24 and 72 h remained fairly constant throughout the incubation period. Fru-2,6-P_2 levels increased between 24 and 96 h of incubation. Recoveries of added metabolites were: ATP, 87 ± 8%; PPi, 84 ± 9%; fru-2,6-P_2, 93 ± 8%. Recoveries were determined by addition of a known quantity of the compound under investigation to the liquid N_2 used to disrupt one set of duplicate samples, and comparing the amounts present in the final extracts. In all cases the amount of

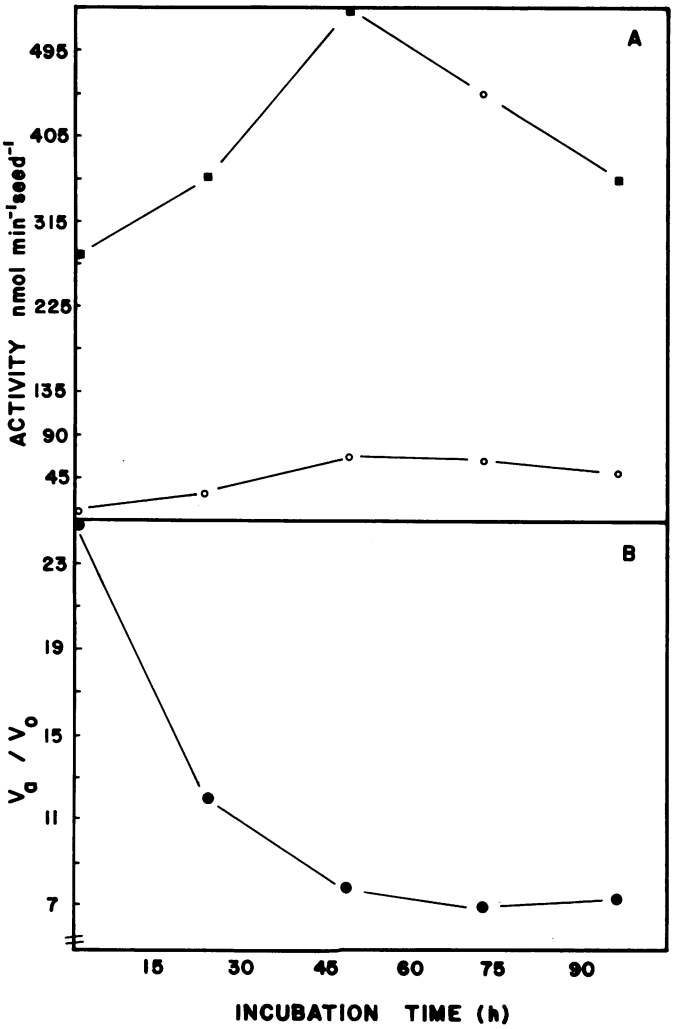

FIG. 2. A, Changes in the total PFP activity in bean seeds during germination at 27°C in the dark. Activity was assayed both in the presence (■) and absence (○) of 10 μM fru-2,6-P_2. B, Changes in the sensitivity of PFP activity to fru-2,6-P_2 during bean seed germination. V_o and V_a represent the enzyme activities measured with and without 10 μM fru-2,6-P_2, respectively.

Table I. *Subcellular Localization of PFP and PFK in Germinating Bean Seeds*

Homogenate from seeds after 72 h of incubation was fractionated by centrifugation. Enzyme activities were measured in the unfractionated homogenate, pellet, and supernatant. All values represent the average of two separate experiments.

Enzyme	Activity in Unfractionated Homogenate	Enzyme Activity	
		Pellet	Supernatant + pellet
	$\mu mol \cdot cm^{-3} \cdot min^{-1}$	*(% of activity in extract)*	
ADH	7.5	0.9	102
PFP	0.38	1.4	98
PFK	0.25	7.9	101
RuBP carboxylase	10.05	20.2	96
Fumarase	14.0	70.1	104

substance added was sufficient to only double the endogenous levels of the compound.

Most of the PFP and PFK activities in the seeds were present in the cotyledons (Table IV). At 48 h of incubation 90% of the total PFK activity, and 80% of the total PFP activity were in the cotyledons. The pattern of change, as well as the total activity of the two enzymes in the cotyledons were similiar during incubation. Changes in the PFP and PFK activity in the cotyledons correlates with changes in the fresh mass of the cotyledons. It is also evident that the activity of both enzymes increases well before radicle emergence.

The PFP activity in the radicle plus hypocotyl is almost 5 times higher than the PFK activity throughout the 96 h incubation period (Table IV). The activity of both enzymes only starts to increase following radicle emergence. The increase in PFP and PFK activity of the radicle plus hypocotyl coincides with an increase in fresh mass and protein content. In the plumule the PFP activity is also much higher than the PFK activity throughout the incubation period, and a very similiar pattern of change in fresh mass, protein content, and enzyme activity is evident.

DISCUSSION

PFP was present at all developmental stages in all the tissues investigated, and in each the activity measured *in vitro* was similar to or higher than that of PFK. This is in contrast to the situation in mung beans where PFP activity is similar to PFK activity only during the first 12 h of incubation (20), and in bean leaves where no PFP activity was detected (16). During the germination of *Phaseolus sp.* active alcohol and lactate fermentation occurs (19, 20, 26) and the obtained results could therefore indicate that PFP participates in glycolysis in bean seeds.

The sensitivity of PFP for fru-2,6-P_2 decreases during germination. Similar changes were observed in germinating mung beans and peas (2, 36). In peas this phenomenom was ascribed to a large and small form of PFP differing in sensitivity towards fru-2,6-P_2 (37). Whether this is the reason for changes in fru-2,6-P_2 sensitivity in beans is not clear. The PFP activities extracted after 48 and 72 h of incubation, elute as a single peak from a Sephacryl S300 column. In both cases the eluted activity is sensitive towards fru-2,6-P_2. In addition, the enzyme apparently remains a single protein of 7.8 S during sucrose density centrifugation in the presence of fru-2,6-P_2 as well as 5 mM PPi (FC Botha, unpublished results).

Previous studies (2, 18, 28) compared total PFP activity to total PFK activity. However, as PFP is localized in the cytosol, competition for the common substrate fru-6-P is most probably restricted to PFP and PFK_c. In beans, PFK_c contributes 60% to the total PFK activity. The kinetic properties of PFK_c of beans at pH 7.5 are similar to those of the PFK_c from the endosperm of developing castor beans (12) but differs largely from that of the PFK from mung beans (2). In the absence of fru-2,6-P_2 PFP, has very low activity. The activation of the enzyme by fru-2,6-P_2 is due to an increase in V_{max} and affinity for both fru-6-P and PPi. The K_m of the bean PFP for fru-6-P is similar to that of the enzyme from peas (36), but is substantially higher than that of several other species (5, 7, 14, 24, 32). In contrast, the K_m value reported for PFP from black gram seeds (2) is much higher than that of bean PFP. The K_m of PFP from beans for fru-6-P is approximately 2 to 3 times higher than that of PFK, even when fully activated by fru-2,6-P_2.

The levels of fru-2,6-P_2 and PPi in beans are similar to those reported for other plant tissues (1, 9, 17, 27, 29). Intracellular compartmentation and an inability to measure the volume of the cytosol accurately, make calculation of the concentrations *in vivo* difficult. If it is assumed that the cytosol is 50% of the total volume of the beans the resulting concentration will be approximately 0.8 to 1.6 μM for fru-2,6-P_2 and 28 to 34 μM for PPi. These concentrations of fru-2,6-P_2 are sufficient to activate PFP and the concentration of PPi is higher than the K_m of PFP for PPi. If the same assumptions are made for fru-6-P and ATP the obtained data suggests that the concentration of fru-6-P may be 30 to 50 μM and that of ATP 180 to 220 μM. The ATP concen-

Table II. *Kinetic Constants of PFP and Cytosolic PFK from Germinating Bean Seeds*

Enzyme	Fru-2,6-P_2	ATP	K_m		V_{max}
			Fru-6-P	PPi	
			μM		$\mu mol \cdot min^{-1}$, mg^{-1} protein
PFK_c	−	46 ± 4	320 ± 40		0.35 ± 0.2
	+	46 ± 4	320 ± 40		0.35 ± 0.2
PFP	−		18100 ± 300	40 ± 6	0.48 ± 0.3
	+		1100 ± 102	25 ± 5	6.50 ± 0.5

Table III. *Levels of ATP, fru-6-P, fru-2,6-P_2 and PPi during Germination of Bean Seeds at 27°C in the Dark*

Each value is the mean ± SD of three separate samples.

Incubation Time	ATP	Metabolite content		
		fru-6-P	fru-2,6-P_2	PPi
h	*nmol·seed*$^{-1}$			
24	85.1 ± 5	11 ± 4	0.182 ± 0.02	7.4 ± 2.1
48	91.8 ± 10	16 ± 5	0.223 ± 0.04	8.9 ± 3.4
72	54.9 ± 12	12 ± 3	0.380 ± 0.06	7.8 ± 2.8

Table IV. *Changes in the Fresh Mass, Total Water Soluble Proteins, and Enzyme Activity in the Cotyledons, Plumule, and Radicle plus Hypocotyl of Bean Seeds during Germination*

Each value represents the mean of measurements from two separately germinated batches of seeds.

Tissue	Incubation Time	Fresh Mass	Protein Content	Enzyme Activity	
				PFP	PFK_c
	h	*mg·embryo*$^{-1}$		*nmol·embryo*$^{-1}$·*min*$^{-1}$	
Cotyledons	2	470	38	250	280
	12	530	44	300	340
	18	580	49	350	380
	24	600	46	360	388
	48	610	30	350	400
	72	490	20	302	300
	96	425	15	245	250
Radicle + hypocotyl	2	10	0.30	28	8
	12	12	0.30	24	9
	18	13	0.32	27	9
	24	15	0.34	25	10
	48	30	0.40	50	12
	72	70	0.82	64	14
	96	142	1.46	93	25
Plumule	2	2.0	0.21	5.5	1.4
	12	2.1	0.22	6.0	1.5
	18	2.3	0.22	5.9	1.4
	24	2.4	0.23	6.4	1.9
	48	2.9	0.26	8.0	2.1
	72	3.0	0.27	8.2	2.3
	96	4.9	0.37	12.0	4.4

tration appears to be much higher than the K_m of PFK_c for ATP. It is, however, evident that even if all the fru-6-P is present in the cytosol, the concentration is well below the K_m of PFP for this substrate. As the K_m value of PFK_c for fru-6-P was approximately 3 times lower than that of PFP, fru-6-P would seem to preferentially bind to the catalytic site of PFK_c. A similar situation is present in germinating mung beans (2). The metabolite content in the different tissues from the bean embryos was not determined as it was impossible to separate them promptly before freeze clamping to avoid rapid changes in metabolite levels.

Recently it was suggested that high PFP activity might be characteristic of tissues where there is extensive biosynthesis (1, 3). It is therefore of particular interest to note that in beans PFP also dominates over PFK activity in the tissues primarily involved in biosynthesis (plumule, radicle and hypocotyl), while PFP activity in the cotyledons, where break down of reserves occurs, is similar to that of PFK activity. The possibility that PFP is responsible for synthesis of PPi needed for the conversion of sucrose to sugar phosphates via sucrose synthase and UDP-glucose pyrophosphorylase, as suggested by Black *et al.* (3), in the growing radicle, hypocotyl and plumule definitely exists.

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