Pyrophosphate:fructose 6-phosphate 1-phosphotransferase and glycolysis in non-photosynthetic tissues of higher plants

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The activity of pyrophosphate:fructose-6-phosphate 1-phosphotransferase [PFK (PP_i); EC 2.7.1.90] in extracts of the storage tissues of leek (*Allium porrum*), beetroot (*Beta vulgaris*) and roots of dandelion (*Lolium temulentum*) exceeded 0.15 $\mu\text{mol/min per g}$ fresh wt. As net flux from fructose 1,6-bisphosphate to fructose 6-phosphate in these tissues is unlikely, it is suggested that PFK (PP_i) does not contribute to gluconeogenesis or starch synthesis. The maximum catalytic activities of PFK (PP_i) in apex, stele and cortex of the root of pea (*Pisum sativum*) and in the developing and the thermogenic club of the spadix of cuckoo-pint (*Arum maculatum*) were measured and compared with those of phosphofructokinase, and to estimates of the rates of carbohydrate oxidation. PP_i and fructose 2,6-bisphosphate in *Arum* clubs were measured. The above measurements are consistent with a glycolytic role for PFK (PP_i) in tissues where there is marked biosynthesis, but not in the thermogenic club of *Arum*. The possibility that PFK (PP_i) is a means of synthesizing pyrophosphate is discussed.

The discovery of pyrophosphate:fructose-6-phosphate 1-phosphotransferase [PFK (PP_i); EC 2.7.1.90] in a wide range of plants (Carnal & Black, 1983; Kruger *et al.*, 1983; Van Schaftingen & Hers, 1983) has led to suggestions that it is glycolytic (Carnal & Black, 1983; Preiss, 1984). Although the available evidence is compatible with this suggestion, much of it is equally compatible with the view that the enzyme catalyses the synthesis of hexose phosphate *in vivo* by converting fructose 1,6-bisphosphate to fructose 6-phosphate. The activity of PFK (PP_i) is readily reversible *in vitro* (O'Brien *et al.*, 1975; Kombrink *et al.*, 1984). The vast majority of plant tissues in which the enzyme has been demonstrated are either photosynthetic (Carnal & Black, 1983) or gluconeogenic (Kruger *et al.*, 1983), so that net flux from fructose 1,6-bisphosphate to fructose 6-phosphate would greatly exceed that in the glycolytic direction.

The aim of the present work was to investigate the role of PFK (PP_i) in plant respiration. Our initial approach was to determine the maximum catalytic activity of PFK (PP_i) in tissue in which

net flux from fructose 1,6-bisphosphate to fructose 6-phosphate is likely to be negligible relative to that in the opposite direction. We also measured PFK (PP_i) in the differentiating root of pea and the developing club of the spadix of *Arum maculatum* (cuckoo-pint) in order to compare the activities with previously published estimates of the activities of PFK (ATP) and the rates of carbohydrate oxidation. In *Arum* we also measured PP_i and Fru-2,6-P₂ in an attempt to establish whether these compounds were present at sufficient concentrations to permit PFK (PP_i) to act glycolytically, and to see if they varied with the rate of glycolysis. We paid particular attention to the difficulties of measuring enzymes and substrates in plant tissues (ap Rees, 1980).

Materials and methods

Materials and plants

Enzymes, cofactors and substrates were from Boehringer Corp. (London), Lewes, East Sussex, U.K., except that PFK (PP_i), fructose 6-phosphate and Fru-2,6-P₂ were from Sigma Chemical Co., Poole, Dorset, U.K.

Mature specimens, variety unknown, of leek (*Allium porrum* L.), onion (*Allium cepa* L.), beetroot (*Beta vulgaris* L. subspecies *vulgaris* var. *rubra*) and

Abbreviations used: PFK (PP_i), pyrophosphate:fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.90); PFK (ATP), 6-phosphofructokinase (EC 2.7.1.11); Fru-2,6-P₂, fructose-2,6-bisphosphate.

sugar beet (*Beta vulgaris* L. subspecies *vulgaris*) were bought locally. We used the white bases of the leaves of leek and onion, and discs, $1\text{ cm} \times 1\text{ mm}$, of the storage tissue of the beets. Seeds of darnel (*Lolium temulentum* L.) were germinated and grown on filter paper, moistened with nutrient solution (Pollock, 1982), in Petri dishes at 20°C with a 10.5h photoperiod and a photon flux of $130\mu\text{mol/s per m}^2$. We used the complete root systems of 6-day-old seedlings. Peas (*Pisum sativum* L. var. Kelvedon Wonder) were grown as described by Edwards *et al.* (1984) and were dissected as described by Wong & ap Rees (1971), except that the stele and cortex were cut to give the regions 6–32 and 32–52mm from the apex. Complete inflorescences of cuckoo-pint (*Arum maculatum* L.) were collected from local natural sites and the swollen end of the appendix of the spadix, the club, was used. The different stages of development of the club were recognized and are defined as described by ap Rees *et al.* (1983). The time between dissection of the first pea root and preparation of the extracts was less than 90min; during this period dissected material was kept at 1°C . All other tissues were extracted within 10min of excision from the parent organ.

Assay of PFK (PP_i)

Tissue (0.15–2.0g fresh wt.) was homogenized, first in a pestle and mortar and then in a ground-glass homogenizer, in 6–30vol. of 75mM-Hepes (pH 7.7)/1mM- MgCl_2 /1mM-EDTA/14mM-2-mercaptoethanol. Examination of the homogenates with a microscope revealed very few unbroken cells. The homogenates, made at 3°C and kept in ice until assayed, were centrifuged at $100000g$ for 30min. The supernatant was assayed at once, except for extracts of *Arum*, which were first passed through a column ($1\text{ cm} \times 13\text{ cm}$) of Sephadex G-25 (coarse grade). The assay, at 25°C , was that described by Kombrink *et al.* (1984). The standard reaction mixture contained, in 1ml: 75mM-Hepes, pH 7.9, 1mM- MgCl_2 , 5mM-fructose 6-phosphate, 0.15mM-NADH, $10\mu\text{M}$ -Fru-2,6- P_2 , 0.6mM- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$, 10units of triosephosphate isomerase, 1unit of glycerol-3-phosphate dehydrogenase and 0.1unit of fructose-bisphosphate aldolase. Optimization led to the following differences: leek, pH 7.7; beetroot, 5units of triosephosphate isomerase; darnel, 1.2mM- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$; *Arum*, pH 7.6. In extracts of *Arum*, PFK (PP_i) was also assayed by measuring the formation of fructose 6-phosphate as described by Kombrink *et al.* (1984). Before use, the coupling enzymes in all the above assays were dialysed for 1h at 4°C against 40mM-glycylglycine, pH 7.0. Protein in the extracts was measured as described by Wong & ap Rees (1971). Optimization of the

enzyme assay comprised variation of the concentration of each component, the pH of the assay mixture, and demonstration that activity was proportional to the amount of extract. Loss of enzyme activity during extraction was investigated by recovery experiments in which we determined the extent to which pure enzyme, added to the extraction medium, was recovered in the final extract, and in recombination experiments in which activity in an extract of a mixture of two tissues was compared with that predicted from measurements made on the separate components of the mixture. In recovery experiments we prepared duplicate samples of tissue and extracted one as described above: we treated the other similarly, except that we added to the extraction medium a measured amount of pure PFK (PP_i) roughly equivalent to that normally found in the homogenate. The difference between the activities found in the two is expressed as a percentage of the added activity to give the recovery. In the recombination experiments we prepared three samples, one of one tissue, one of the other, and one that was a mixture of equal weights of the two tissues.

Assay of metabolites

For assay of PP_i , single clubs of *Arum* were freeze-clamped as described by ap Rees *et al.* (1977a), and then killed, extracted and assayed as described by Edwards *et al.* (1984). For measurement of Fru-2,6- P_2 , single clubs were freeze-clamped, dropped into liquid N_2 , pulverized, left at 20°C until the N_2 had almost evaporated, and then covered with 2–4ml of 50mM-NaOH at 80°C . This suspension was kept at 80°C for 5min, cooled on ice and centrifuged at $27000g$ for 2min. The sediment was washed with 50mM-NaOH ($2 \times 1\text{ ml}$), and the washings and the original supernatant were combined and adjusted to pH 7.8 with ice-cold 1M-acetic acid in 20mM-Hepes. Fru-2,6- P_2 in the neutralized extract was assayed by measuring its ability to stimulate pure PFK (PP_i) (Van Schaftingen & Hers, 1983). The assay mixture, 1.0ml, contained: 50mM-Tris, adjusted to pH 8.0 with acetic acid, 2mM-magnesium acetate, 0.14mM-NADH, 0.85mM-fructose 6-phosphate, 0.13unit of fructose-bisphosphate aldolase, 0.85unit of glycerol-3-phosphate dehydrogenase, 2.5units of triosephosphate isomerase, 0.005unit of PFK (PP_i) and 0.5mM- $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$. The enzymes had been dialysed against 40mM-glycylglycine, pH 7.0, at 4°C for 60min and made 0.2% (w/v) with respect to bovine serum albumin. To check the specificity of the assay (Sener *et al.*, 1984), we showed for every extract that there was negligible stimulation after the extract had been incubated in 0.1M-HCl at 20°C for 5min. In the

recovery experiments, single clubs were split lengthwise into halves; one half was extracted as above, the other was treated similarly, except that measured amounts of PP_i (10–20 nmol) or Fru-2,6- P_2 (10–20 pmol) were added immediately after freeze-clamping.

The statistical significance of differences between mean values was assessed by Student's *t* test.

Results and discussion

PFK (PP_i) in non-photosynthetic, non-gluconeogenic, non-starchy tissues

Synthesis of starch from translocated sucrose may involve conversion of some of the sucrose into triose phosphates for entry into the amyloplast (Macdonald & ap Rees, 1983). Thus appreciable net flux from fructose 1,6-bisphosphate to fructose 6-phosphate in tissues of higher plants is least likely in those that contain little starch and are neither photosynthetic nor gluconeogenic. The storage tissues of leeks, onions (Parkin, 1899), sugar beet and beetroot (Giaquinta, 1979), and the roots of dandelion (Smith, 1973), fall into this category. Extracts of each of these tissues showed appreciable activity (>40 nmol/min per g fresh wt.) of PFK (PP_i). After optimization, the rates for dandelion roots, the white base of leek leaves and beetroot storage tissue were found to be 1.36 ± 0.06 , 0.88 ± 0.14 , 0.15 ± 0.01 μ mol/min per g fresh wt. respectively. Each value is the mean $\pm$ S.E.M. for six extracts; recovery of added PFK (PP_i) was more than 90% for each tissue. These values are high relative to the rates of respiration of plant tissues (James, 1953): that for beetroot is about four times that needed to account for the CO_2 production of freshly cut tissue (Bennet-Clark & Bexon, 1943). The occurrence of such substantial activities of PFK (PP_i) in the above tissues makes it very unlikely that this enzyme catalyses a net synthesis of fructose 6-phosphate from fructose 1,6-bisphosphate *in vivo*.

PFK (PP_i) during differentiation of pea root

In order to investigate whether PFK (PP_i) is involved in carbohydrate oxidation, we determined its maximum catalytic activity during the differentiation of the pea root. We dissected the apical 52 mm of the root to give regions at different stages of differentiation. The general properties of these regions, or regions closely comparable with them, and the reliability of the dissection have been described (Fowler & ap Rees, 1970; Wong & ap Rees, 1971). The assay was optimized for each region of the root. Recovery of pure PFK (PP_i), added to the buffer used to extract the undissected

apical 52 mm of the roots, was essentially complete (99%).

Regardless of the stage of differentiation, PFK (PP_i) activity (Table 1) was substantial and almost certainly exceeded the rate of carbohydrate oxidation *in vivo*. CO_2 production (nmol/min per mg of protein) has been measured for the following regions of the root: apical 6 mm, 54 (Smith, 1978); stele (6–26 mm from apex), 30; stele (26–46 mm), 40; cortex (6–26 mm), 36; cortex (26–46 mm), 36 (Wong & ap Rees, 1971). The relationship between rates of CO_2 production and carbohydrate oxidation is complicated by sequestration of respiratory intermediates for biosynthesis (ap Rees, 1980). The manner in which pea roots metabolize [^{14}C]glucose (Fowler & ap Rees, 1970) suggests that no more than 50% of the label that entered the respiratory pathways was used in biosynthesis. Thus the assumption that each mol of hexose that entered the respiratory pathways produced 3 mol of CO_2 would give a generous estimate of the rate of carbohydrate oxidation. Division of the rates of CO_2 production by 3 gives values that are well below the activities of PFK (PP_i) reported in Table 1.

The maximum catalytic activity of PFK (PP_i) varies considerably during the differentiation of the pea root. The high activity in the apex is largely maintained in the cells that differentiate into stele, but diminishes markedly during differentiation into cortex. The activity of PFK (PP_i) does not show any obvious correlation with that of PFK (ATP) or carbohydrate oxidation. The absolute activity of PFK (PP_i), and that relative to PFK (ATP), are greatest in the stele and apex. This

Table 1. Activities of PFK (PP_i) and PFK (ATP) in different regions of the root of *Pisum sativum*

PFK (PP_i) was measured in roots of 6-day-old seedlings after dissection as described in the Materials and methods section. Values for PFK (ATP) for the apex are from Fowler & ap Rees (1970); those for stele and cortex refer to 6–26 and 26–46 mm from the apex and are from Wong & ap Rees (1971). Values are means $\pm$ S.E.M. for the numbers of samples shown in parentheses. For PFK (PP_i), $P > 0.05$ for apex versus stele (6–32), stele (6–32) versus stele (32–52); for all other comparisons between different regions of the root for this enzyme, $P < 0.05$.

Region of root	Enzyme activity (nmol/min per mg of protein)	
	PFK (PP_i)	PFK (ATP)
Apical 6 mm	297 $\pm$ 9 (6)	95 $\pm$ 3 (7)
6–32 mm from apex		
Stele	259 $\pm$ 33 (5)	46 $\pm$ 2 (10)
Cortex	136 $\pm$ 9 (5)	52 $\pm$ 2 (10)
32–52 mm from apex		
Stele	200 $\pm$ 25 (6)	50 $\pm$ 2 (5)
Cortex	66 $\pm$ 10 (6)	46 $\pm$ 3 (5)

suggests that PFK (PP_i) may be particularly important in tissues in which biosynthesis is most marked.

PFK (PP_i), Fru-2,6-P₂ and PP_i in *Arum* club

We examined the above hypothesis by measuring PFK (PP_i), Fru-2,6-P₂ and PP_i during the development of the club of *Arum maculatum*, and relating these measurements to our previous estimates of PFK (ATP) and the rate of carbohydrate oxidation. This club passes through a number of arbitrarily defined, but readily recognizable and well characterized, stages of development that culminate in a sudden brief period of extremely rapid and thermogenic oxidation of carbohydrate (ap Rees, 1977). We call the successive stages α , β , γ , prethermogenesis and thermogenesis. The first four are marked by cell expansion, net synthesis of protein, massive accumulation of starch, and extensive incorporation of label from [¹⁴C]glucose into cellular material. Thermogenesis is characterized by the rapid rise in respiration, rapid breakdown of starch and protein, and extensive conversion of [¹⁴C]glucose into ¹⁴CO₂ (Lance, 1972; ap Rees *et al.*, 1976). Thus from α -stage to prethermogenesis the metabolism of the club is dominated by biosynthesis, whereas at thermogenesis there is a rapid switch to almost complete catabolism.

The assays for PFK (PP_i) were optimized at α -stage and thermogenesis; no differences were found in the optimal conditions. Estimates of recovery of pure enzyme were: α -stage, 96%; prethermogenesis, 117%; thermogenesis, 116%. Recombination experiments between α - and β -stages, γ -stage and prethermogenesis, thermogenesis and γ -stage gave values for the mixture that were within 10% of those predicted from measurements made on the separate components. A similar pattern of results was obtained when PFK (PP_i) was assayed, after optimization, in the direction of fructose 6-phosphate formation.

We were unable to demonstrate any change in the maximum catalytic activity of PFK (PP_i) during the development of the club (Table 2). Previously, we (ap Rees *et al.*, 1976) used measurements of CO₂ production and the patterns of [¹⁴C]glucose metabolism to estimate that clubs at α -stage, β -stage and prethermogenesis oxidized carbohydrate at 0.19, 0.24 and 0.31 μ mol of hexose/min per g fresh wt. respectively. The data in Table 2 show that from α -stage to prethermogenesis the clubs contain sufficient PFK (PP_i) to catalyse the rate of carbohydrate oxidation *in vivo*. At thermogenesis the rate of starch breakdown can be measured and is a direct measure of glycolysis (ap Rees *et al.*, 1977b). In clubs still attached to the plant, an average rate of glycolysis of 7.6 μ mol of hexose/min per g fresh wt. was observed over a 30 min period just before thermogenesis. As the rate of respiration was rising rapidly during the 30 min, it is likely that the maximum rate of glycolysis was near 10 μ mol/min per g fresh wt. Both of these estimates are well in excess of the maximum catalytic activity of PFK (PP_i) at thermogenesis. These values suggest that, at thermogenesis, PFK (PP_i) could make, at the most, a relatively minor contribution to glycolysis. This view is reinforced by comparison of PFK (PP_i) with PFK (ATP) and other glycolytic enzymes (ap Rees *et al.*, 1976; Bulpin & ap Rees, 1978). The maximum catalytic activities of the latter rise markedly and continuously throughout development of the club to values well in excess of the rate of glycolysis at thermogenesis. Thus the ratio PFK (PP_i)/PFK (ATP) changes from 4.9:1 at α -stage to 0.14:1 at thermogenesis.

Estimates of the recovery of added PP_i were: α -stage, 90%; prethermogenesis, 99%; thermogenesis, 94%. The corresponding values for Fru-2,6-P₂ were 117, 83 and 106%. No PP_i or Fru-2,6-P₂ was detected in extracts treated with yeast inorganic pyrophosphatase or subjected to acid hydrolysis respectively. Three aspects of the measurements of

Table 2. Activities of PFK (PP_i) and PFK (ATP), and contents of PP_i and Fru-2,6-P₂ during the development of the club of *Arum maculatum*

Single clubs at each stage of development were assayed: values are means $\pm$ S.E.M. for the numbers of clubs shown in parentheses. Values for PFK (ATP) are from ap Rees *et al.* (1976), but are expressed per g fresh wt. Except for club wt., differences between the mean and that of the preceding stage are indicated by: no superscript, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$; **** $P < 0.001$.

Stage of development	Club wt. (mg)	Enzyme activity (μ mol/min per g fresh wt.)		Metabolite content (nmol/g fresh wt.)	
		PFK (PP _i)	PFK (ATP)	PP _i	Fru-2,6-P ₂
α	239 $\pm$ 8 (29)	5.60 $\pm$ 0.21 (9)	1.15 $\pm$ 0.09 (5)	21.1 $\pm$ 1.8 (9)	0.56 $\pm$ 0.02 (6)
β	616 $\pm$ 18 (25)	5.24 $\pm$ 0.42 (6)	3.76 $\pm$ 0.73 (6)**	36.9 $\pm$ 2.7 (7)****	0.36 $\pm$ 0.03 (6)****
γ	1169 $\pm$ 38 (26)	4.12 $\pm$ 0.30 (7)	12.28 $\pm$ 1.80 (9)***	33.2 $\pm$ 3.9 (8)	0.30 $\pm$ 0.03 (6)
Pre-thermogenesis	1346 $\pm$ 74 (24)	4.02 $\pm$ 0.60 (6)	18.15 $\pm$ 1.40 (9)*	27.5 $\pm$ 3.4 (6)	0.31 $\pm$ 0.05 (6)
Thermogenesis	1205 $\pm$ 107 (31)	4.26 $\pm$ 0.58 (10)	30.01 $\pm$ 3.05 (7)***	36.2 $\pm$ 3.0 (9)	0.46 $\pm$ 0.08 (7)

metabolites (Table 2) are stressed. First, there is appreciable Fru-2,6- P_2 in clubs at each stage of development. Even the highest estimate of the cytosolic volume gives a concentration *in vivo* that is greater than that required to give optimum activity of PFK (PP_i) *in vitro* (Kombrink *et al.*, 1984). Second, at each stage of development clubs contain PP_i in amounts comparable with those of glycolytic intermediates. At α -stage there are 36.4 and 20 nmol per g fresh wt. of fructose 6-phosphate and fructose 1,6-bisphosphate, respectively: the corresponding values at thermogenesis are 43.7 and 77.1 (ap Rees *et al.*, 1977a). Intracellular compartmentation and an inability to measure the volume of the cytosol make calculation of the concentrations *in vivo* difficult. If it is assumed that the cytosol is 10% of the volume of the club and that PP_i is confined to the cytosol, the data in Table 2 suggest that the concentration of PP_i may be 0.2 to 0.4 mM *in vivo*. If the same assumptions are made for fructose 6-phosphate and fructose 1,6-bisphosphate, the free energy change for PFK (PP_i) (Kruger *et al.*, 1983) can be used to calculate the concentration of P_i that would be present in the cytosol if the reaction catalysed by PFK (PP_i) were at equilibrium *in vivo*. Values of 1.4 and 0.74 mM are obtained for α -stage and thermogenesis respectively. These are a little lower than the recent estimate of 6 mM for non-photosynthetic cells of higher plants (Rebeille *et al.*, 1983). Our data provide no evidence that the reaction catalysed by PFK (PP_i) is markedly displaced from equilibrium *in vivo* and are consistent with it catalysing a near-equilibrium reaction. Finally, the fact that neither PP_i nor Fru-2,6- P_2 showed any detectable change between pre-thermogenesis and thermogenesis adds to the evidence that PFK (PP_i) does not contribute appreciably to glycolysis at thermogenesis. The data for *Arum* are consistent with the view that PFK (PP_i) contributes most to metabolism during biosynthesis and least during thermogenesis.

General comments

The results of the recovery and recombination experiments, and the optimization of the assays, strongly suggest that our estimates of enzymes and metabolites reflect the activities and amounts present *in vivo*. Recently, it has been argued that PFK (ATP) from rabbit muscle (Wong *et al.*, 1984) and the cytosol of spinach leaves (Balogh *et al.*, 1984) can be converted reversibly into PFK (PP_i) *in vitro*. This argument has been seriously challenged (Gancedo, 1984). The following is evidence that such interconversion did not occur during our extraction and assay of PFK (PP_i). First, recovery of added PFK (PP_i) and PFK (ATP) was good. Secondly, activities of both enzymes in the extracts

at 2°C remained constant for up to 1 h. Thirdly, when a sample of pea roots was freeze-clamped and dropped straight into extraction buffer, the activity of PFK (PP_i) was the same as that found in a duplicate sample that had been homogenized and extracted in the usual way. Finally, the activities of PFK (PP_i) and PFK (ATP) varied independently, not inversely, during the development of the *Arum* club and pea root.

Our results do not implicate PFK (PP_i) in the net conversion of triose phosphate into fructose 6-phosphate during either gluconeogenesis or starch synthesis. The presence of PP_i in pea roots (Edwards *et al.*, 1984) and in *Arum* clubs, and the evidence that PFK (PP_i) catalyses a near-equilibrium reaction in the latter give some support for a glycolytic role for PFK (PP_i). This view is strengthened by our demonstration that, in most of the tissues that we examined, there is enough PFK (PP_i) to mediate glycolysis. However, this is not always so: our data strongly suggest that PFK (PP_i) makes little contribution to glycolysis in *Arum* clubs at thermogenesis. In general, our measurements show that the behaviour of PP_i and Fru-2,6- P_2 , and the maximum catalytic activity of PFK (PP_i), do not correlate closely with the rate of carbohydrate oxidation. However, high activities of PFK (PP_i) were shown to be characteristic of tissues in which there is extensive biosynthesis: the apex and stele of pea root, and the developing club of *Arum*. Thus PFK (PP_i) may contribute to glycolysis primarily in tissues dominated by biosynthesis and the use of respiratory intermediates for the synthesis of cellular material. Alternatively, PFK (PP_i) may not contribute to glycolysis at all, but be a means of synthesizing PP_i . Such synthesis could be controlled by Fru-2,6- P_2 and produce fructose 6-phosphate that would be promptly converted into fructose 1,6-bisphosphate in glycolysis. PP_i formed in this way might be needed, *inter alia*, for the conversion of sucrose into hexose phosphates via sucrose synthase (EC 2.4.1.13) and UDP-glucose pyrophosphorylase (EC 2.7.7.9). Each of the tissues that we showed to have high activities of PFK (PP_i) is characterized by extensive biosynthesis from incoming sucrose.

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References

- ap Rees, T. (1977) *Symp. Soc. Exp. Biol.* **31**, 7–32
- ap Rees, T. (1980) in *The Biochemistry of Plants* (Davies, D. D., ed.), vol. 2, pp. 1–29, Academic Press, London and New York
- ap Rees, T., Fuller, W. A. & Wright, B. W. (1976) *Biochim. Biophys. Acta* **437**, 22–35

- ap Rees, T., Fuller, W. A. & Wright, B. W. (1977a) *Biochim. Biophys. Acta* **461**, 274–282
- ap Rees, T., Wright, B. W. & Fuller, W. A. (1977b) *Planta* **134**, 53–56
- ap Rees, T., Bryce, J. H., Wilson, P. M. & Green, J. H. (1983) *Arch. Biochem. Biophys.* **227**, 511–521
- Bennet-Clark, T. A. & Bexon, D. (1943) *New Phytol.* **42**, 65–92
- Balogh, A., Wong, J. H., Wotzel, C., Soll, J., Cséke, C. & Buchanan, B. B. (1984) *FEBS Lett.* **169**, 287–292
- Bulpin, P. V. & ap Rees, T. (1978) *Phytochemistry* **17**, 391–396
- Carnal, N. W. & Black, C. C. (1983) *Plant Physiol.* **71**, 150–155
- Edwards, J., ap Rees, T., Wilson, P. M. & Morrell, S. (1984) *Planta* **162**, 188–191
- Fowler, M. W. & ap Rees, T. (1970) *Biochim. Biophys. Acta* **201**, 33–44
- Gancedo, J. M. (1984) *FEBS Lett.* **175**, 369–370
- Giaquinta, R. T. (1979) *Plant Physiol.* **63**, 828–832
- James, W. O. (1953) *Plant Respiration*, p. 21, Oxford University Press, Oxford
- Kombrink, E., Kruger, N. J. & Beevers, H. (1984) *Plant Physiol.* **74**, 395–401
- Kruger, N. J., Kombrink, E. & Beevers, H. (1983) *FEBS Lett.* **153**, 409–412
- Lance, C. (1972) *Ann. Sci. Nat. Bot. Biol. Vég.* **12^{ème} Ser.** **13**, 477–495
- Macdonald, F. D. & ap Rees, T. (1983) *Biochim. Biophys. Acta* **755**, 81–89
- O'Brien, W. E., Bowien, S. & Wood, H. G. (1975) *J. Biol. Chem.* **250**, 8690–8695
- Parkin, J. (1899) *Philos. Trans. R. Soc. London Ser. B* **191**, 35–80
- Pollock, C. J. (1982) *New Phytol.* **90**, 645–650
- Preiss, J. (1984) *Trends Biochem. Sci.* **9**, 24–27
- Rebeille, F., Bligny, R., Martin, J.-B. & Douce, R. (1983) *Arch. Biochem. Biophys.* **225**, 143–148
- Sener, A., Van Schaftingen, E., Van de Winkel, M., Pipeleers, D. G., Malaisse-Lagae, F., Malaisse, W. J. & Hers, H.-G. (1984) *Biochem. J.* **221**, 759–764
- Smith, A. M. (1978) Ph.D. Thesis, University of Cambridge
- Smith, D. (1973) in *Chemistry and Biochemistry of Herbage* (Butler, G. W. & Bailey, R. W., eds.), vol. 1, pp. 105–155, Academic Press, London and New York
- Van Schaftingen, E. & Hers, H.-G. (1983) *FEBS Lett.* **164**, 195–200
- Wong, W. J. & ap Rees, T. (1971) *Biochim. Biophys. Acta* **252**, 296–304
- Wong, J. H., Balogh, A. & Buchanan, B. B. (1984) *Biochem. Biophys. Res. Commun.* **121**, 842–847