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METABOLISM OF UDP-GLUCOSE BY DEVELOPING EMBRYOS OF ROUND AND WRINKLED VARIETIES OF *PISUM SATIVUM*

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Key Word Index—*Pisum sativum*; Leguminosae; pea; embryos; UDP-glucose; enzymes; pyrophosphate; fructose-2,6-bisphosphate.

Abstract—Developing embryos from growing pods of round (Birte) and wrinkled (Greenshaft) varieties of *Pisum sativum* were studied during the period of rapid growth. No significant activity of UDP-glucose phosphorylase or of pyrophosphohydrolases for ATP or UTP was detected in extracts of embryos. Estimates of the maximum catalytic activities of UDP-glucose pyrophosphorylase and pyrophosphate:fructose 6-phosphate 1-phosphotransferase [PFK(PPi)] showed them to change during development roughly in parallel with that of sucrose synthase, and to exceed the maximum estimates of the rate of sucrose breakdown. Measurements of the embryo content of UTP, UDP-glucose, inorganic pyrophosphate, hexose monophosphates and fructose-1,6-bisphosphate suggest that the reactions catalysed by sucrose synthase, UDP-glucose pyrophosphorylase and PFK(PPi) approach equilibrium *in vivo*. Fructose-2,6-bisphosphate content showed a positive correlation with the rate at which the embryos converted sucrose to starch. Incubation of embryos in [U-^{14}C]sucrose led to substantial labelling of UDP-glucose. It is suggested that sucrose synthase makes a major contribution to sucrose breakdown by forming UDP-glucose that is converted to glucose 1-phosphate by UDP-glucose pyrophosphorylase using pyrophosphate generated by PFK(PPi).

INTRODUCTION

Measurements of the relative activities of acid, and alkaline, invertase and sucrose synthase (EC 2.4.1.13) suggest a major role for the latter in the immediate metabolism of the sucrose delivered to the developing embryo of pea [1]. Although sucrose synthase can use nucleoside diphosphates other than UDP, this enzyme forms UDP-glucose preferentially when UDP is available [2]. Sucrose synthase is located in the cytosol, as is UDP-glucose pyrophosphorylase [3]. As the latter is one of the major enzymes that use UDP it seems most likely that there will be sufficient UDP in the cytosol to ensure that UDP-glucose is the product of sucrose synthase *in vivo*. The fate of this UDP-glucose is not established. About 75% of the [^{14}C]sucrose metabolized by developing embryos of peas was recovered in starch and compounds derived from respiratory intermediates [1]. As ADP-glucose rather than UDP-glucose appears to be the precursor of starch in non-photosynthetic tissues [4], it seems that much of the UDP-glucose formed by sucrose synthase must be converted to hexose monophosphate for further metabolism. Present evidence suggests two possible routes, the UDP-glucose phosphorylase reported by Gibson and Shine [5] to be present in potato tubers, and UDP-glucose pyrophosphorylase (EC 2.7.7.9). Use of the latter would require a supply of inorganic pyrophosphate (PPi). This could be provided either by the ATP:pyrophosphohydrolase reported from maize by Echeverria and Humphreys [6], or by the widely distributed [7] cytosolic enzyme pyrophosphate:fructose 6-phosphate 1-phosphotransferase [PFK(PPi), EC 2.7.1.90].

The aim of the work described in the present paper was to discover if any of the above hypotheses was correct. We estimated the maximum catalytic activities of the enzymes, and compared these with our previous [1] estimates of the rates of sucrose breakdown. For the enzymes found in appreciable activity we also determined the ratio of tissue content of products to substrates, the mass action ratio. Comparison of this ratio with the equilibrium constant of the reaction indicates the degree to which the reaction *in vivo* approaches equilibrium, and thus provides evidence of the direction of net flux. We made these measurements on developing embryos from growing pods of two varieties of pea, Birte and Greenshaft, henceforth called round and wrinkled, respectively, in which we have already studied the mechanism of sucrose partitioning [1]. We took developing seeds from growing pods and removed the testa. The whole of the material within the testa, which at the stages of development studied, consisted almost entirely of cotyledons together with a small radicle, was used without further dissection. As in our previous work, the measurements were made during the period of rapid growth of the embryo, and the developmental stages are defined according to embryo fresh weight, as 100, 200, 300 and 400 mg. As we could not always obtain embryos of these precise weights, for each stage we took embryos that were within 40 mg of the specified weight. For example, for the 100 mg stage we used embryos between 60 and 140 mg; for each sample within this range values for enzyme activity or metabolite content are expressed per 100 mg to give the value at the 100 mg stage.

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RESULTS

Measurement of enzymes

For UDP-glucose phosphorylase we assayed extracts of mixtures of both varieties of embryo, each at the 250–350 mg stage, and extracts of mature tubers of potato (cv. Desirée). We were unable to detect activity in extracts of either the embryos or the potato. We used two methods to assay for this enzyme. In the first, we followed the procedure of Gibson and Shine [5] and measured release of [^{14}C]glucose 1-phosphate from UDP[^{14}C]glucose. For the second assay, unfractionated extracts were passed through Sephadex G-25 and then incubated in the reaction mixture used for the first assay except that the UDP-glucose was unlabelled. After 15 min the incubation was stopped and activity was assessed by determining hexose monophosphate production from measurements of glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate. The treatment with Sephadex reduced the amount of hexose monophosphate in the extract taken for each assay to less than 2 nmol. From Table 1 it can be seen that such hexose phosphate production that we did detect varied appreciably in comparable samples, and was not dependent upon added phosphate, or UDP-glucose or fructose-2,6-bisphosphate.

The assay for UDP-glucose pyrophosphorylase was optimized in respect of the concentration of the components, and the pH, of the assay mixture. Further, activity was shown to be linearly related to the amount of extract assayed. Extremely high activities were found at all stages of development (Table 2). No differences were found between the two varieties and activity was shown to rise markedly during embryo development.

We examined extracts of mixtures of both types of pea embryos (each embryo at the 250–350 mg stage), clubs of the spadix of *Arum maculatum* at γ stage [8], and scutella from maize seeds that had been germinated for 4 days for both ATP and UTP pyrophosphohydrolase activity (Table 3). Each extract was incubated with ATP or UTP plus UDP-glucose and UDP-glucose pyrophosphorylase so that production of PPi would give rise to glucose 1-phosphate. After a 30 min incubation the reaction was stopped and the amounts of glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate were measured to give total hexose monophosphate produced (Table 3). We established that the hexose monophosphate content of the amount of extract added to each sample was less than 1 nmol, and that no hexose monophosphates could be detected in reaction mixtures that had been incubated in the absence of extract.

Extracts of all three tissues showed some apparent activity in the assay for ATP pyrophosphohydrolase (Table 3). However, this activity was not dependent upon ATP, was only partially dependent upon UDP-glucose, and essentially unaffected by fructose-2,6-bisphosphate at a concentration said to stimulate activity in maize extracts [6]. The extracts of pea, *Arum* and maize contained, respectively, 6.4, 17.5 and 7.0 nmol of glucose plus fructose per 100 μl taken for each assay. These amounts correspond closely to the amounts of hexose phosphates found in reaction mixtures incubated with ATP and without UDP-glucose. Thus we attribute the latter to the activity of hexokinase in the extracts. We also detected hexose formation in the assay for UTP-pyrophosphohydrolase. Again this was not affected by 1 μM fructose-2,6-bisphosphate, and, except in pea extracts, was higher in the absence of UTP than in its presence. The stimulation by UTP in pea extracts is attributed to the fact that we found that the UTP added to the reaction mixture was contaminated with 20 nmol inorganic pyrophosphate.

The assays for PFK(PPi) and phosphofructokinase [PFK(ATP); EC 2.7.1.11] were optimized and checked as described for UDP-glucose pyrophosphorylase. We carried out recombination and recovery experiments to discover whether differential losses of activity occurred during extraction. In the former we assayed three samples,

Table 1. Assay of extracts of developing embryos of peas and of potato tubers for UDP-glucose phosphorylase

Tissue	Alterations to standard assay	Activity (nmol hexose phosphate formed min^{-1} g ft. wt)			
		+ Fru-2,6-P_2		− Fru-2,6-P_2	
		Assay I*	Assay II	Assay I	Assay II
Pea embryos	None	—	21	66.1	21
	No KH_2PO_4	10.4	—	—	8
	20 mM KH_2PO_4	15.5	—	—	—
	No UDP-glucose	—	—	—	21
	0.56 mM UDP-glucose	14.8	—	—	—
	2.2 mM UDP-glucose	25.9	—	—	—
Potato tuber	None	0.9	44	0.7	40
	No KH_2PO_4	1.8	—	1.3	17
	5 mM KH_2PO_4	1.5	—	0.8	—
	20 mM KH_2PO_4	1.5	—	0.9	—
	No UDP-glucose	—	—	—	54
	0.56 mM UDP-glucose	0.3	—	0.3	—
	1.1 mM UDP-glucose	0.9	—	0.7	—

*Assays I and II measured release of [^{14}C]glucose 1-phosphate and hexose monophosphate production, respectively.

Table 2. Estimates of UDP-glucose pyrophosphorylase, PFK(PPi) and PFK(ATP) in developing embryos of round and wrinkled peas

Enzyme	Variety	Enzyme activity (μmol min^{-1} embryo^{-1}) in embryos of fr. wt			
		100	200	300	400 mg
UDP-glucose pyrophosphorylase	Round	4.2±0.7	12.1±1.5	24.7±1.5	30.2±2.4
	Wrinkled	2.2±0.4	13.0±2.2	17.2±1.6	38.7±3.7
PFK(ATP)	Round	0.030±0.004	0.145±0.014	0.254±0.024	0.421±0.010
	Wrinkled	0.033±0.010	0.093±0.007	0.270±0.026	0.378±0.021
PFK(PPi)	Round	0.180±0.009	0.335±0.025	0.529±0.012	0.586±0.017
	Wrinkled	0.133±0.013	0.202±0.011	0.464±0.032	0.553±0.030

Values are means ± s.e.m. of estimates from at least four samples.

Table 3. Assay of extracts of pea embryos, club of γ stage spadix of *Arum maculatum*, and maize scutellum for ATP and UTP pyrophosphohydrolase activity

Substrate	Components omitted from reaction medium	Activity (nmol hexose phosphate formed/ 100 μl extract)		
		Pea	*Arum*	Maize
ATP	None	20.1	30.4	26.7
	ATP	14.5	30.1	31.5
	UDP-glucose	9.7	19.4	7.2
	Fru-2,6-P_2	20.9	24.3	23.7
UTP	None	56.3	24.1	21.5
	UDP-glucose	3.2	5.8	1.9
	UTP	14.5	30.1	31.5
	Fru-2,6-P_2	57.1	22.9	21.2

one of embryos of round peas, one of embryos of wrinkled peas, and one that was a mixture of equal weights of both types of embryo. The activities of PFK(PPi) and PFK(ATP) in the mixtures were, respectively, 110 and 121% of those predicted from the measurements made on the separate components of the mixtures. In recovery experiments, measured amounts of pure enzyme, comparable to the activity found in the tissue extracts, were added to the extraction medium and their recovery in the final extract was determined. Values of 118 and 132%, respectively, were obtained for PFK(PPi) and PFK(ATP). The data in Table 2 show that substantial activities of both enzymes were found at all the stages of development examined. No differences between the two varieties were found and the activities of both enzymes rose markedly during development. At all stages the activity of PFK(PPi) was appreciably greater than that of PFK(ATP).

Measurements of substrates

For each compound we carried out recovery experiments designed to measure losses during killing and extraction of the embryos. For each test we prepared duplicate samples, each a mixture of the two types of embryo. One sample was freeze-clamped, extracted and assayed in the usual way; the other was treated similarly except that a measured amount of the compound was added to the freeze-clamped sample before it was killed. The amount added was similar to that normally detected in the sample of embryos. Comparison of the amounts found in the two extracts allowed estimation of the recovery of the added compound.

We assayed UDP-glucose by isolating it by HPLC and measuring A_{254}. The following is evidence that the peak was due to UDP-glucose. First the material from the extracts had a retention time that was within 0.2 min of that of authentic UDP-glucose (32.85 min). ADP-glucose was the only detectable component of the extracts with a retention time (26.02 min) comparable to that of UDP-glucose; even so these two compounds are well separated by this method [4]. Acid hydrolysis of the peak attributed to UDP-glucose followed by re-analysis by HPLC led to the complete disappearance of the original peak. Assay of the uridine and glucose in these hydrolysates showed that for round peas the amounts were 75 and 73%, respectively, of those expected if the peak was due to UDP-glucose. The corresponding figures for wrinkled peas were 77 and 76%.

Recoveries for fructose-2,6-bisphosphate were 117 ± 10% and 105 ± 8% (means ± s.e.m. of six estimates) for embryos of round and wrinkled peas, respectively. Appreciable amounts of fructose-2,6-bisphosphate were found in the embryos of both varieties at all stages of development examined (Fig. 1). After the 200 mg stage there was significantly more ($P < 0.01$) in the round than in the wrinkled peas. Amounts per embryo increased during development. This increase was greater in round than in wrinkled peas: in the former the content per g fresh weight rose from 690 pmol at the 100 mg stage to 1415 pmol at the 400 mg stage. The corresponding figures for wrinkled peas were 490 and 290 pmol.

In order to establish the equilibrium position of the reactions *in vivo* we measured metabolites in embryos at the 300–400 mg stage of development (Table 4). We were unable to measure PPi in extracts of embryos of round peas because addition of the extracts to the assay mixture caused a precipitate to form. Recoveries of all the other compounds were satisfactory. Embryos of round peas contained more UTP ($P < 0.01$) and UDP-glucose ($P < 0.01$) than did embryos of wrinkled peas. For all the other compounds measured the reverse was true ($P < 0.001$ except for glucose 1-phosphate where it was < 0.01).

We investigated whether UDP-glucose became labelled when embryos were incubated in [U-^{14}C]sucrose. We did

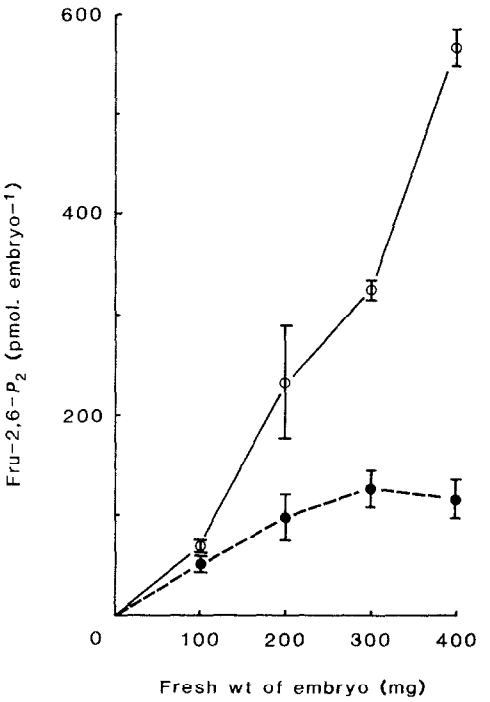

Fig. 1. Fructose-2,6-bisphosphate content of developing embryos of round (○) and wrinkled (●) peas. Each value is the mean of estimates from at least four embryos: bars represent s.e.m. where these are large enough to be shown.

this by incubating embryos at the 300 mg stage in [U-^{14}C]sucrose and then isolating the UDP[^{14}C]glucose by HPLC and determining its specific activity. Recovery of ^{14}C from the HPLC column was at least 98%. The following is evidence that the peak attributed to UDP[^{14}C]glucose was not significantly contaminated by other labelled intermediates that have retention times somewhat similar to UDP-glucose. First, analysis of a mixture of UDP[^{14}C]glucose, ADP[^{14}C]glucose and [^{14}C]glucose 1-phosphate gave complete separation and 100% recovery of each compound. Second, treatment of the extract with alkaline phosphatase prior to analysis, a treatment that hydrolyses the common phosphate esters, did not diminish the labelling of the peak attributed to UDP[^{14}C]glucose. From Table 5 it can be seen that [^{14}C]sucrose readily labelled UDP-glucose in both varieties of embryo. In brief incubations the specific activity of the UDP[^{14}C]glucose was 12% of the supplied [^{14}C]sucrose, this figure rose to 20–38% in the longer incubations.

DISCUSSION

We argue that the optimization of the enzyme assays, and the recombination and recovery experiments, authenticate our estimates of enzymes and substrates. Our inability to detect appreciable activities of UDP-glucose phosphorylase and either ATP or UTP pyrophosphohydrolase is unlikely to be due to extensive loss of such enzymes during extraction as our recovery experiments in this and our previous paper [1] show that pea embryos are particularly amenable material for extraction and measurement of enzymes. The lack of demonstrable activity of UDP-glucose phosphorylase and the pyrophosphohydrolase, together with the high activities of the other enzymes that we measured, lead us to conclude that the former enzymes play no appreciable role in the sucrose metabolism of the developing embryo of peas.

Pea embryos are characterized by very high activities of UDP-glucose pyrophosphorylase (Table 2). These are much greater than expected of a tissue that is breaking down rather than synthesizing sucrose, and that is diverting no more than 8% of metabolized [^{14}C]sucrose into structural polysaccharides [1]. The maximum estimates of the rate at which the developing embryos metabolize sucrose are 118 and 52 nmol min^{-1} g^{-1} fr. wt, respectively, for round and wrinkled peas [1]. The values we obtained for the activities of UDP-glucose pyrophosphorylase, PFK(PPi) (Table 2) and sucrose synthase [1] are well in excess of these estimates. In general, all three enzymes show similar increases in activity during the development of the embryo and these coincide with the period of rapid growth and rapid metabolism of sucrose [1]. The measurements are therefore consistent with the hypothesis that sucrose synthase makes a major contribution to sucrose breakdown to produce UDP-glucose that is metabolized to glucose 1-phosphate by UDP-glucose pyrophosphorylase using PPi made by PFK(PPi).

The above view requires fructose-2,6-bisphosphate to be present in sufficient concentrations for PFK(PPi) to function. It seems likely that fructose-2,6-bisphosphate is

Table 4. Measurements of sugar phosphates in developing embryos of round and wrinkled peas

Compound	Content (nmol g^{-1} fr. wt)		Recovery of added compound (%)
	Round	Wrinkled	
UDP-glucose	421 ± 15 (8)	324 ± 23 (8)	86 ± 1 (6)
PPi	Not determined	9 ± 1 (11)	90 ± 3 (6)
UTP	351 ± 34 (7)	175 ± 15 (7)	88 ± 7 (6)
Glc-1-*P*	31 ± 3 (6)	70 ± 4 (6)	92 ± 9 (6)
Glc-6-*P*	442 ± 26 (7)	1253 ± 39 (7)	91 ± 3 (3)
Fru-6-*P*	92 ± 5 (7)	283 ± 14 (7)	89 ± 8 (3)
Fru-1,6-P_2	26 ± 4 (7)	54 ± 7 (8)	90 ± 4 (10)
Triose phosphates	20 ± 2 (7)	55 ± 4 (8)	113 ± 3 (6)

Values are means ± s.e.m. from the number of samples shown in parentheses.

Table 5. Specific activity of UDP[^{14}C]glucose isolated from embryos of round and wrinkled peas that had been incubated in 0.19 M [U-^{14}C]sucrose

	Round		Wrinkled	
Time in [^{14}C]sucrose (hr)	3	18	3	18
Specific activity of [^{14}C]sucrose supplied (dpm nmol^{-1})	47	81	105	104
Specific activity of UDP[^{14}C]glucose (dpm nmol^{-1})*	6±0	12±2	31±4	20±1

*Values are means ± s.e.m. of at least three estimates.

confined to the cytosol [7]. If we assume that the cytosol is 10% of the tissue volume then the maximum and minimum concentrations of fructose-2,6-bisphosphate in the developing embryos are 2.9 and 14.2 μM. These are so far above the concentration needed to give optimal activity of PFK(PPi) *in vitro* [9] as to suggest that a significant proportion of the fructose-2,6-bisphosphate is bound to protein *in vivo* as has been reported for rat hepatocytes [10]. There seems little doubt that pea embryos contain sufficient fructose-2,6-bisphosphate to allow PFK(PPi) to function.

Our considerations of mass-action ratios concentrate on the embryos of wrinkled peas as we lack data on PPi for round peas. In our calculations we have assumed that the compounds listed in Table 4 are largely confined to the cytosol. We appreciate that a portion of many of the intermediates is located in the amyloplast but suggest that the small volume of the stroma in these plastids means that this proportion is small [4]. For sucrose synthase we estimate the UDP content of the embryos from the measurements of UTP (Table 4), and the previously reported equilibrium constant of nucleoside-diphosphate kinase of 0.91 [11] and the ATP/ADP ratio of 2.7 [12]. Values of 143 and 71 nmol g^{-1} fr. wt are obtained for embryos of round and wrinkled peas, respectively. Stickland and Wilson [13] have shown that developing embryos of Birte and Greenshaft contain 0.93 and 2.8 μmol fructose g^{-1} fr. wt, and 60 and 120 μmol sucrose g^{-1} fr. wt, respectively. We use our estimates of UDP and UDP-glucose to calculate the ratio of sucrose to fructose that would exist in the cytosol, where sucrose synthase is located [3], if the reaction catalysed by this enzyme is at equilibrium *in vivo*. Published values of K range from 0.149 [14] to 0.625 [15]: the lower value would require ratios of sucrose to fructose in the cytosol of 19.6 and 30.3 for embryos of round and wrinkled peas, respectively. For the higher value, the corresponding ratios are 4.7 and 7.3. Given the high overall ratio of sucrose to fructose in the embryos, the presence of fructokinase in the cytosol [16], and the fact that sucrose is delivered to the cytosol, it seems very likely that sucrose synthase in both types of embryo catalyses a net flux from sucrose to UDP-glucose and fructose.

For UDP-glucose pyrophosphorylase, phosphoglucomutase and glucose 6-phosphate isomerase the similarity between the apparent equilibrium constants and the mass-action ratios (Table 6) is good evidence that all three reactions are close to equilibrium *in vivo*. We use the free energy change for PFK(PPi) [19] and our estimates of PPi, fructose-1,6-bisphosphate and fructose 6-phosphate (Table 4) to calculate the concentration of Pi that would have to exist in the cytosol of the embryos of wrinkled peas if the PFK(PPi) reaction were at equilibrium. We have assumed that the cytosol is 10% of the total volume, and that the above compounds are confined to the cytosol. A value of 1.7 mM is obtained. Current estimates of the cytosolic concentration of Pi [20] suggest values of 6 mM. Thus, in pea embryos, as in the club of *Arum maculatum* [8], the PFK(PPi) reaction appears to be sufficiently close to equilibrium *in vivo* for it to be a means of generating PPi.

The data in this and our previous paper [1] suggest that sucrose breakdown in developing peas is mediated, at least in part, by sucrose synthase, to give UDP-glucose that is subsequently metabolized by UDP-glucose pyrophosphorylase at the expense of PPi generated by PFK(PPi). The key enzymes are present in sufficient activity. Their maximum catalytic activities vary in the expected way during embryo development, and the equilibrium positions of the reactions *in vivo* are consistent with the proposal. Further support for our view is provided by the labelling of UDP[^{14}C]glucose by [^{14}C]sucrose *in vivo*. When the likely dilution of the [^{14}C]sucrose by endogenous sucrose is taken into account, the specific activity of the UDP[^{14}C]glucose may be regarded as high compared to that of the supplied [^{14}C]sucrose. The fact that in the

Table 6. Comparison of apparent equilibrium constants and mass-action ratios in developing embryos of round and wrinkled peas

	K'			Mass-action ratio	
Reaction	Value	Reference	Reactants	Round	Wrinkled
UDP-glucose pyrophosphorylase	2.9–3.6	[17]	$\frac{[UTP]\,[Glc\text{-}1\text{-}P]}{[UDP\text{-}Glc]\,[PPi]}$	—	4.2
Phosphoglucomutase	19	[18]	[Glc-6-*P*]:[Glc-1-*P*]	14.4	17.9
Glucose 6-phosphate isomerase	0.36–0.47	[18]	[Fru-6-*P*]:[Glc-6-*P*]	0.21	0.23

longer incubations the specific activity of the UDP[^{14}C]glucose from round peas was double that from wrinkled peas (Table 5) correlates closely with our evidence that the round embryos metabolized sucrose at twice the rate shown by the wrinkled embryos [1]. Finally, this difference in the rates of sucrose metabolism also correlates with the significantly higher content of fructose-2,6-bisphosphate of the embryos of round peas (Fig. 1). This suggests that fructose-2,6-bisphosphate contributes to the regulation of sucrose metabolism by controlling the production of PPi needed to metabolize the UDP-glucose formed by sucrose synthase. A comparable situation may exist in developing tubers of potato [21].

EXPERIMENTAL

Materials. Substrates, enzymes and cofactors were from Boehringer except that UDP-glucose, fructose 6-phosphate, glucose-1,6-bisphosphate, fructose-2,6-bisphosphate, UTP and PFK(PPi) were from Sigma. Isotopes were from the Radiochemical Centre, Amersham. Seeds of *Pisum sativum* L., cv. Birte (J1 1068 round-seeded) and cv. Greenshaft (J1 430 wrinkled-seeded) were generously supplied by the John Innes Institute, Norwich. Peas were grown and embryos were harvested as in ref. [1]. Clubs of *Arum maculatum* L. were obtained as described in ref. [8]. Scutella were obtained from seeds of *Zea mays* L. cv. Golden Bantam that had been germinated for 4 days as described for peas in ref. [8].

Enzyme assays. Plant material was homogenized as in ref. [1]. For PFK(PPi) and PFK(ATP) the extraction medium was 60 mM HEPES, pH 7.7, 1 mM $MgCl_2$: for the other enzymes it was 50 mM glycylglycine, pH 7.5, except for UDP-glucose phosphorylase. For the latter we used two procedures: the first was that described in ref. [5] except for the substitution of Sephadex G-50 (Coarse) for Bio-Gel P-10, the second was homogenization as for the other enzymes but in 50 mM glycylglycine, pH 7.5, 2 mM dithiothreitol. Except for the first method for UDP-glucose phosphorylase, the above homogenates were centrifuged at 100 000 *g* for 30 min and the supernatant was assayed directly [PFK(ATP), UDP-glucose pyrophosphorylase] or after passage through a column of Sephadex G-25 (Coarse) [PFK(PPi), UDP-glucose phosphorylase (second method), pyrophosphohydrolase]. Enzymes were measured at 25° in the following reaction mixtures and according to the accompanying refs: UDP-glucose pyrophosphorylase [17], 80 mM glycylglycine, pH 8.0, 1 mM $Na_4P_2O_7$, 1 mM $MgCl_2$, 4.8 μM glucose-1,6-bisphosphate, 0.4 mM NAD, 4 units phosphoglucomutase, 1.4 units glucose-6-phosphate dehydrogenase (EC 1.1.1.49, NAD dependent from *Leuconostoc*), 0.8 mM UDP-glucose in 1 ml: PFK(PPi) [8], 75 mM HEPES, pH 7.6, 7.5 mM fructose 6-phosphate, 1 mM $MgCl_2$, 0.15 mM NADH, 1 μM fructose-2,6-bisphosphate, 1 unit fructose-1, 6-bisphosphate aldolase (EC 4.1.2.13), 1 unit triosephosphate isomerase (EC 5.3.1.1),1 unit glycerol-3-phosphate dehydrogenase (EC 1.1.1.8), 0.3 mM $Na_4P_2O_7$ in 1.0 ml: PFK(ATP) [22], 50 mM glycylglycine, pH 8.2, 0.25 mM ATP, 0.1 mM NADH, 2 mM $MgCl_2$, 0.27 unit fructose-1,6-bisphosphate aldolase, 0.06 unit triosephosphate isomerase, 0.7 unit glycerol-3-phosphate dehydrogenase, 10 mM fructose 6-phosphate in 3 ml: UDP-glucose phosphorylase, preparations made as in ref. [5] were incubated in 10 mM HEPES, pH 7.0, 10 mM KH_2PO_4, 1.1 mM UDP[U-^{14}C]glucose (30 Ci mol^{-1}), 1 μM fructose-2,6-bisphosphate, 1 mM dithiothreitol in 0.15 ml. The reaction was started with extract, incubation was for 15 min, after which 75 μl HCl-activated charcoal was added, the mixture was then centrifuged at 11 000 *g* for 2 min and ^{14}C in the supernatant was measured: for the second assay of UDP-glucose phosphorylase, assay conditions were similar except that UDP-glucose was used and the supernatant obtained after centrifugation at 11 000 *g* was assayed for glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate as described in the next section. For ATP and UTP pyrophosphohydrolase [6] the reaction mixture contained in 1 ml: 80 mM Tris–HCl, pH 8.0, 10 mM ATP or UTP, 5 mM UDP-glucose, 1 μM fructose-2,6-bisphosphate, 2 mM $MgCl_2$, 0.3 mM $CaCl_2$, 1 mM NaF. The mixture was incubated at 25° for 30 min, heated to 100° for 1 min, cooled and assayed for glucose 1-phosphate, glucose 6-phosphate and fructose 6-phosphate as described below.

Substrate assays. Samples of 0.3–1.0 g fr. wt of 1–3 embryos were freeze-clamped [23] within 2 min of taking the embryos out of the pod and 6 min of picking the pod. Fructose-2,6-bisphosphate was assayed as in ref. [8]. For all other substrates the freeze-clamped material was dropped into 1 ml 1.41 M $HClO_4$ at 2° in a microfuge tube, homogenized, kept on ice for 30 min (15 min for UTP), and then centrifuged at 11 000 *g* for 2 min. The sediment was re-extracted with 0.5 ml H_2O and centrifuged again. The two supernatants were combined, brought to pH 7–8 with 5 M K_2CO_3 and centrifuged to remove the $KClO_4$. The resulting supernatant was assayed for substrates as in the following refs and reaction mixtures: hexose monophosphates [24], 0.3 M TEA, pH 7.5, 3 mM $MgSO_4$, 12 mM NAD, 0.39 ml extract, 2.8 units glucose 6-phosphate dehydrogenase (NAD specific from *Leuconostoc*), and, successively, 7 units of glucose 6-phosphate isomerase and 2.8 units of phosphoglucomutase: fructose-1,6-bisphosphate [25], 30 mM imidazole-HCl, pH 7.0, 0.1 mM NADH, 0.9 unit fructose-1,6-bisphosphate aldolase, 1 unit triosephosphate isomerase, 1 unit glycerol 3-phosphate dehydrogenase, 0.6 ml extract: UTP [26], 272 mM glycylglycine, pH 8.7, 1.6 mM NAD, 4.3 mM EDTA, 0.065 unit UDP-glucose dehydrogenase (EC 1.1.1.22), 0.1 ml extract: 0.96 unit UDP-glucose pyrophosphorylase was added once all the UDP-glucose in the extract had been oxidized. PPi was measured as in ref. [27] and hexose as in ref. [21]. The methods for the measurements of UDP-glucose by HPLC, and the methods used in the authentication of these measurements are in ref. [4].

General. Labelling of UDP-glucose by [^{14}C]sucrose was determined by incubating samples of 2 or 3 embryos (each of between 250 and 350 mg fr. wt) in 2 ml 10 mM Mes, pH 5.0, 0.19 M [U-^{14}C]sucrose (sp. act. varied as in Table 5) at 25° in the dark. At the end of the incubation the embryos were blotted dry, freeze-clamped and then extracted and fractionated by HPLC as described for the assay of UDP-glucose. ^{14}C was measured as in ref. [1].

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