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The relationship between the rate of starch synthesis, the adenosine 5′-diphosphoglucose concentration and the amylose content of starch in developing pea embryos

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Abstract. Mutations that reduced the rate of starch synthesis in pea (*Pisum sativum* L.) embryos through effects on enzymes on the pathway from sucrose to adenosine 5′-diphosphoglucose (ADPglucose) also led to a reduction in the amylose content of the starch of developing embryos. Evidence is presented that this relationship between rate of synthesis and the composition of starch is due to the fact that amylopectin-synthesising isoforms of starch synthase have higher affinities for ADPglucose than the amylose-synthesising isoform. First, developing mutant embryos (*rb*, *rug3* and *rug4* mutants) displayed both reduced amylose contents in their starches and reduced ADPglucose contents relative to wild-type embryos. Second, incubation of detached, wild-type embryos for 6 h at high and low glucose concentrations resulted in differences in both ADPglucose content and the relative rates of amylose and amylopectin synthesis. At 0.25 M glucose both ADPglucose content and the proportion of synthesised starch that was amylose were about twice as great as at 25 μM glucose. Third, $S_{0.5}$ values for soluble (amylopectin-synthesising) starch synthases in developing embryos were several-fold lower than that for granule-bound (amylose synthesising) starch synthase. Estimates of the expected amylose contents of the starch of the mutant embryos, based on the reduction in their ADPglucose contents and on the $S_{0.5}$ values of the starch synthases, were very similar to the measured amylose contents. The implications of these results for the determination of starch composition are discussed.

Key words: Adenosine 5′-diphosphoglucose – Amylose – Embryo (pea) – *Pisum* (starch synthesis) – Starch synthase – Starch synthesis

Introduction

In pea embryos and in the unicellular green alga *Chlamydomonas*, mutations affecting enzymes involved in the synthesis of ADPglucose, the substrate for synthesis of starch, reduce both the overall rate of starch synthesis and the ratio of amylose to amylopectin in the starch (Kooistra 1962; Lloyd et al. 1996; Van den Koornhuyse et al. 1996; Wang et al. 1998). There is a general relationship between the extent of reduction in the rate of starch synthesis and the extent of reduction of the amylose content of the starch. Mutations at the *rug4*, *rug3* and *rb* loci of peas lie in genes encoding sucrose synthase (Craig et al. 1999), plastidial phosphoglucomutase (Harrison et al. 1998) and ADPglucose pyrophosphorylase (Hylton and Smith 1992; R. Burton and C. Martin, John Innes Centre: personal communication) respectively. Mutations at the *rb* locus reduce the starch content of the mature seed (about 50% of the dry weight in the wild-type) by about 40%, and amylose content of the starch (about 30% in the wild-type) is reduced by up to 25%, depending upon which mutant allele is present at the *rb* locus. In *rug3* mutants, the starch content of mature seeds is only 1–12% of the dry weight. The amylose content of the starch is reduced by 60% relative to the wild-type in lines carrying the least severe allele (*rug3-a*). In *rug4* mutants, starch content is reduced by 20%, and reported reductions in amylose content of the starch range from zero to 15% (Lloyd 1995; Wang et al. 1998). In *Chlamydomonas*, mutations at the *sta1* and *sta5* loci reduce the starch content by about 90% or more due to their effects on ADPglucose pyrophosphorylase and plastidial phosphoglucomutase, respectively (Ball et al. 1991; Van den Koornhuyse et al. 1996). Whereas the storage starch of wild-type *Chlamydomonas* contains 15–30% amylose (Buléon et al. 1997), starch from the mutants contains no detectable amylose.

It is not obvious why mutations directly affecting ADPglucose synthesis should affect the composition of starch as well as the rate of its synthesis. These mutations do not appear to reduce the activity of granule-bound starch synthase I (GBSSI), the isoform of

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Abbreviations: ADPglucose = adenosine 5′-diphosphoglucose, GBSSI = granule-bound starch synthase I

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starch synthase responsible for amylose synthesis, or to have major effects on other starch synthases or on starch-branching enzymes which are collectively responsible for amylopectin synthesis. In developing embryos of *rb* and *rug4* mutants, the maximum catalytic activities of soluble and granule-bound starch synthase and starch-branching enzyme are comparable to those in wild-type embryos (Smith et al. 1989; Craig et al. 1999). In developing *rug3* embryos, total starch synthase activity is higher than in wild-type embryos and GBSSI protein is present at levels comparable to or higher than those in wild-type embryos (Harrison 1996; Clarke 1998). The *sta1* mutant of *Chlamydomonas* has the same activities of starch synthase and starch-branching enzyme as wild-type *Chlamydomonas* (Ball et al. 1991). The activities of these enzymes in the *sta5* mutant have not been reported.

The simplest explanation of the effects of these mutations on the amylose content of starch is that they result from reduced ADPglucose concentrations in the plastid (Martin and Smith 1995; Van den Koornhuyse et al. 1996). If the affinity for ADPglucose of GBSSI is lower than that of the soluble starch synthases responsible for amylopectin synthesis, a reduced ADPglucose concentration might well reduce the rate of amylose synthesis to a greater extent than the rate of amylopectin synthesis.

A second possible explanation of the effects of the mutations on the amylose content of starch is that they are mediated by changes in the concentrations of malto-oligosaccharides in the plastid. Amylose synthesis by GBSSI in isolated starch granules from pea embryos requires malto-oligosaccharides in addition to ADPglucose (Denyer et al. 1996). If the levels of these compounds were lower in mutant than in wild-type embryos, this might account for at least part of the difference in the amylose contents of the starches.

We have investigated whether either of these explanations can account for the relationship between the rate of starch synthesis and the amylose content of the starch in pea embryos. First, we compared the ADPglucose and malto-oligosaccharide contents and the amylose-to-amylopectin ratios of developing wild-type embryos with those of *rb*, *rug4* and *rug3-a* mutants. Second, we manipulated the ADPglucose contents of detached, wild-type embryos by incubating them with different amounts of metabolisable substrate, and examined the effects on the relative rates of amylose and amylopectin synthesis. Third, we estimated the affinities of granule-bound and soluble starch synthases for ADPglucose, and compared these $S_{0.5}$ values with estimates of plastidial ADPglucose concentrations.

Materials and methods

Plant material. Wild-type peas were of the BC3 line described by Hedley et al. (1994). All mutant lines were in this or a very similar background, and were as described by Hedley et al. (1994) for *rb*, by Harrison et al. (1998) for *rug3* (the *rug3-a* line), and by Craig et al. (1999) for *rug4* (the *rug4-b* line). Plants were grown in a greenhouse at a minimum temperature of 12 °C under natural light, supplemented with artificial light at approximately 250 μmol quanta photosynthetically active radiation $\cdot\ m^{-2} \cdot s^{-1}$ between October and March. Plants were permitted to develop one pod at each of three nodes, after which the apex and any side shoots were removed.

Measurement of ADPglucose and malto-oligosaccharides. Immediately after harvest an embryo was removed from one of the central seeds of the pod and rapidly freeze-clamped between two pieces of laboratory film (ap Rees et al. 1977). Subsequent steps were at 0–4 °C. The tissue was homogenised in a mortar in 1 M perchloric acid (approx 4 ml), incubated for 30 min, then centrifuged. The supernatant was neutralised to pH 7 with 0.4 M Hepes, 2 M KOH, 0.4 M KCl and centrifuged. The supernatant was assayed for metabolites.

For measurement of ADPglucose, the extract was freeze-dried and resuspended in 1 ml water. After centrifugation at 10 000 g for 15 min, 20-μl samples were subjected to high-pressure liquid chromatography according to Jenner (1991) on a p10-SAX anion-exchange column (HiChrom, Reading, Berks, UK). The identity of the putative ADPglucose peak was confirmed by chromatography of mixtures of extracts and authentic nucleotides and sugar nucleotides, and by acid hydrolysis (Jenner 1991). The ADPglucose content of samples was calculated by comparison of the peak area with those from known amounts of pure ADPglucose. In recovery experiments (see *Results*), the percentages of ADPglucose recovered were: wild-type, 93 ± 2 (13); *rb*, 112 ± 14 (3); *rug3*, 122; *rug4*, 84. For wild-type and *rb*, values are means ± SE from the number of individual experiments given in parentheses. For *rug3* and *rug4*, values are means from two experiments.

For measurement of malto-oligosaccharides, 0.2 ml extract at pH 6 was incubated with 70 units *β*-fructosidase (yeast; Boehringer, Lewes, Sussex, UK) at 25 °C for 1 h, then with 30 mM Hepes (pH 7.5), 2.5 mM ATP, 2.4 mM NAD, 1 mM $MgCl_2$, 3 units hexokinase, 4 units glucose 6-phosphate dehydrogenase (*from Leuconostoc mesenteroides*; Boehringer) in a final volume of 1 ml at 25 °C for 1 h. After heating to 100 °C for 2 min, the mixture was adjusted to pH 6 and incubated with 4 units transglucosidase (Megazyme International, Bray, Wicklow, Ireland) and 0.5 units α-amyloglucosidase (*Aspergillus niger*; Boehringer) at 25 °C for 1 h. The mixture was treated with 5 mg activated charcoal, centrifuged at 10 000 g for 10 min, and assayed enzymatically for glucose (Lowry and Passonneau 1972). In recovery experiments (see *Results*) the percentages of maltose recovered were: wild-type, 107 ± 11 (10); *rb*, 118 ± 18 (5); *rug4*, 95%. For wild-type and *rb*, values are means ± SE from the number of individual experiments given in parentheses. For *rug4* the value is the mean from two experiments.

Extraction and analysis of starch. Individual embryos were extracted in ethanol, autoclaved and assayed for starch (Stitt et al. 1978). Samples containing known amounts of starch were dried, resuspended in 1 M NaOH at 1 ml per 20 mg starch, incubated at 25 °C for 30 min, diluted 2-fold with water and heated to 90 °C for 2 min. The resulting suspension was subjected to Sepharose CL2B chromatography on 5-ml columns (0.5 mg starch per column), according to Denyer et al. (1995). One fraction of 0.2 ml was collected per minute. Individual fractions were adjusted to pH 6, incubated with 4 units each of α-amyloglucosidase (from *Aspergillus niger*; Boehringer) and α-amylase (from porcine pancreas; Boehringer), and assayed for glucose (Lowry and Passonneau 1972). Fractions in the first and second peaks of carbohydrate were assumed to contain amylopectin and amylose, respectively.

Incubation and analysis of detached embryos. Incubations were in 50-ml flasks, each containing three, freshly harvested embryos and 4 ml 10 mM Mops (pH 7.2) and either 0.25 M glucose or 25 μM glucose and 0.25 M mannitol. After 2 h in the dark at 25 °C with gentle shaking, [U-^{14}C]glucose was added to give 30 MBq $\cdot$ mol^{-1} in flasks with 0.25 M glucose and 30 GBq $\cdot$ mol^{-1} in flasks with 25 μM glucose. After a further 4 h, embryos from each flask were

Table 1. Adenosine diphosphoglucose contents of developing pea embryos. The content of ADPglucose was measured in extracts of developing embryos harvested from greenhouse-grown wild-type and mutant plants either between January and March (Winter) or between June and August (Summer). Values are means ± SE of the number of independent measurements shown in parentheses, except where two independent measurements are shown. Each measurement was made on an embryo from a different pod, and for each genotype pods were taken from at least nine different plants

Genotype	ADPglucose content (nmol · embryo^{-1})			
	200–400 mg FW		400–600 mg FW	
	Summer	Winter	Summer	Winter
Wild-type	12.8 ± 6.4 (5)	11.9 ± 1.3 (9)	33.6 ± 2.9 (7)	17.9 ± 1.5 (18)
rb	1.9 ± 0.4 (9)	5.0 ± 1.4 (7)	6.4 ± 0.6 (6)	7.0 ± 0.9 (14)
rug3	2.1 ± 0.5 (7)	2.8 ± 0.6 (5)	5.2 ± 2.6	5.5 ± 1.0 (14)
rug4	6.3 ± 0.9 (7)	7.4 ± 1.5 (11)	17.6 ± 1.4 (3)	5.5 ± 0.7 (11)

extracted together in a mortar with a total of 5 ml of 100 mM Tris (pH 7.0), 0.5 M NaCl, 1 mM EDTA, 1 mM dithiothreitol at 4 °C, filtered through two layers of Miracloth, stirred for 30 min and centrifuged at 10 000 g for 10 min. The pellet was washed twice in each of 20 g · l^{-1} aqueous SDS, water, and acetone (at −20 °C), then dried in air. The incorporation of ^{14}C into amylose and amylopectin was determined by solubilisation of starch followed by Sepharose CL2B chromatography as described above. Embryos for measurement of ADPglucose were incubated as described above, except that no [^{14}C]glucose was added.

Measurement of starch synthase activity. For granule-bound activity, starch was extracted from embryos and assayed according to Denyer et al. (1995). For soluble activity, embryos were extracted and assayed according to Craig et al. (1998). Estimates of the affinity for ADPglucose are expressed as $S_{0.5}$, the concentration at which the rate was half-maximal. This reflects that fact that, at least for the granule-bound activity, the value is unlikely to reflect the true K_m

Results

Relationship between ADPglucose content and starch composition in mutant and wild-type embryos. The ADPglucose contents of extracts of developing embryos of the wild-type and *rug4*, *rb* and *rug3* mutant lines were assayed by HPLC. Embryos were freeze-clamped immediately after harvest and extracted in perchloric acid. Evidence that amounts of ADPglucose were not altered by these procedures was provided by recovery experiments in which known amounts of ADPglucose, comparable to those in the tissue samples, were added to one of two replicate samples of tissue at the time of freeze-clamping. The percentage of the added ADPglucose recovered was routinely in the range 85–120% (see *Materials and methods*).

The amount of ADPglucose per embryo was lower in the three mutant lines than in the wild-type (Table 1). This was true during both the earlier and the later part of the main period of starch synthesis (embryos of 200–400 and 400–600 mg fresh weight, respectively), and when plants were grown in winter and in summer. The ADPglucose contents of *rb* and *rug3* embryos were 15–40% of those of wild-type embryos, whereas contents of *rug4* embryos were 30–60% of those of wild-type embryos. All of these differences were statistically significant (Student's *t* test, 95% confidence limit).

In order to assay malto-oligosaccharides, glucose and compounds that might give rise to it were first enzymically removed from neutralised perchloric-acid extracts. Malto-oligosaccharides were then converted to glucose by incubation with glucosidases and assayed enzymatically. Recoveries of maltose, assessed as described for ADPglucose above, were in the range 95–118% (see *Materials and methods*).

Amounts of glucose in malto-oligosaccharides were similar in wild-type, *rug4* and *rb* embryos. For embryos of 200–400 mg fresh weight, malto-oligosaccharide contents were 1.1 ± 0.3(8), 1.1 ± 0.3(8) and 1.3 ± 0.3(9) μmol glucose equivalents per embryo for wild-type, *rb* and *rug4* embryos, respectively (values are means ± SE of measurements on the number of individual embryos given in parentheses). For embryos of 400–600 mg fresh weight these values were 3.2 ± 0.4(20), 3.0 ± 0.5(7) and 6.1 ± 0.8(14) μmol per embryo. Since the very high levels of sugars other than malto-oligosaccharides in developing *rug3* embryos (Harrison 1996) rendered measurement of glucose in malto-oligosaccharides inaccurate, values for this genotype are not presented.

Amylose and amylopectin in solubilised samples of starch were separated by gel-permeation chromatography (Denyer et al. 1995). In the earlier part of the main period of starch synthesis (embryos of 250–450 mg fresh weight), there were no statistically significant differences between the amylose contents of the starch of wild-type, *rug4* and *rb* embryos, but the starch of *rug3* embryos contained significantly less amylose than the starch of wild-type embryos ($P < 0.05$; Table 2). In the later part of the main period of starch synthesis (embryos of 450–650 mg fresh weight), the amylose content of the starch of both *rb* and *rug3*, but not *rug4*, embryos was lower than that of wild-type embryos. Taken over the whole of the developmental period examined, the amylose contents of starch of all three mutants were statistically significantly lower than that of the wild-type ($P < 0.01$, 0.001 and 0.05 for *rb*, *rug3* and *rug4*, respectively).

Content of ADPglucose and relative rates of amylose and amylopectin synthesis in detached, wild-type embryos. To provide a further, independent means of examining the relationship between ADPglucose content and relative rates of synthesis of amylose and amylopectin in developing embryos, we incubated the detached embryos of

Table 2. Amylose content of the starch of developing pea embryos. Starch was solubilised from water-insoluble material extracted from developing pea embryos, and amylose and amylopectin were separated by gel-permeation chromatography on a column of Sepharose CL2B. The glucan contents of fractions from the column were estimated after conversion to glucose. The glucan in the amylose peak is expressed as a fraction of the glucan recovered from the column. Values are means ± SE of measurements made on the numbers of individual embryos shown in parentheses. Each embryo was from a different pod, and for each genotype pods were taken from at least three plants, from the same batch as those used for the winter measurements of ADPglucose in Table 1. The recovery of glucan from the column was estimated for each sample by expressing the total glucan in the eluted fractions as a percentage of that applied to the column. Values (mean ± SE of the number of samples shown in parentheses) were: wild-type, 87 ± 15% (9); *rb*, 120 ± 7% (9); *rug3*, 94 ± 13% (9); *rug4* 110 ± 22% (10)

Genotype	Amylose content of starch (%)	
	250–450 mg FW	450–650 mg FW
Wild-type	26 ± 2 (3)	27 ± 3 (6)
rb	22 ± 3 (4)	15 ± 1 (3)
rug3	15 ± 3 (4)	13 ± 3 (5)
rug4	21 ± 4 (5)	20 ± 3 (6)

Table 3. Adenosine diphosphoglucose contents and amylose synthesis in developing wild-type embryos incubated in high and low concentrations of glucose. Samples of three freshly harvested embryos were incubated for a total of 6 h in media that contained either 25 μM glucose or 0.25 M glucose. [^{14}C]Glucose was added to some samples after 2 h of incubation. At the end of the incubation, non-radioactive samples were extracted and assayed for ADPglucose. Water-insoluble material was extracted from radioactive samples, starch was solubilised from this material, and amylose and amylopectin were separated by gel-permeation chromatography on a column of Sepharose CL2B. Radioactivity in the amylose peak is expressed as a percentage of that recovered from the column. Values are means ± SE of measurements from four separate experiments. To check for losses of ADPglucose during extraction and assay, recovery experiments were carried out with known amounts of ADPglucose as described in *Results*. Recoveries were 89 ± 22% for embryos incubated in 25 μM glucose and 81 ± 8% for embryos incubated in 0.25 M glucose (mean ± SE of values from three separate experiments). The recovery of radioactivity from the column was estimated for each sample, by expressing the total ^{14}C in the eluted fractions as a percentage of that applied to the column. Values (mean ± SE from four samples of starch) were: 25 μM glucose, 87 ± 4%; 0.25 M glucose, 85 ± 3%

Concentration of glucose in the medium	ADPglucose content ($nmol^{-1} \cdot embryo^{-1}$)	Radioactivity in amylose as a percentage of that in starch
0.25 M	15.1 ± 2.6	35 ± 0.8
25 μM	5.3 ± 1.5	16 ± 0.6

wild-type peas in media containing two different concentrations of glucose. From previous reports we expected that the rate of starch synthesis, and hence the level of ADPglucose, would be maintained at in vivo levels in high concentrations of exogenous sugar, whereas the rate of starch synthesis and the level of ADPglucose would fall at low concentrations. Edwards and colleagues reported that detached pea embryos supplied with high concentrations of sugar had linear rates of uptake over about 8 h and patterns of partitioning between starch synthesis and other metabolic pathways similar to that which occurs in vivo (Edwards and ap Rees 1986). In detached embryos incubated in a medium containing no sugars, the ADPglucose content declined by about 40% in 2 h (Edwards et al. 1988).

After 6 h incubation in 0.25 M glucose, the ADPglucose contents of wild-type pea embryos were comparable to those in freshly harvested embryos (Table 3). In contrast, ADPglucose contents fell by 65% over this period when the medium contained only 25 μM glucose. To examine the relative rates of amylose and amylopectin synthesis at the two glucose concentrations, embryos were supplied with [^{14}C]glucose after an initial 2-h incubation, and incubated for a further 4 h. The amylose fraction of starch from embryos incubated at the higher glucose concentration contained 35% of the ^{14}C in starch, whereas at the lower glucose concentration the amylose contained only 16% of the ^{14}C in starch (Table 3). This difference is highly statistically significant ($P < 0.001$).

We checked whether the proportion of ^{14}C in amylose changed with time. Any movement of ^{14}C between the amylose and amylopectin fractions or differential loss of ^{14}C from these fractions with time would affect interpretation of the distribution of label after a 6-h incubation. It was found that the amylose fraction contained the same proportion of the ^{14}C in starch regardless of the length of the incubation. When replicate samples of embryos were incubated for either 2 h or 7 h at the lower glucose concentration, amylose accounted for 20 and 25% of the label in starch after 2 h, and 19 and 20% after 7 h (values from two separate incubations, each of three embryos, for each length of incubation). Values at 2 h and 7 h were also comparable at the higher glucose concentration.

We investigated whether the osmotic potential of the incubation affected the relative rates of amylose and amylopectin synthesis. The incubation medium containing 25 μM glucose also contained 0.25 M mannitol, so that at the start of the incubation, the osmotic potential of this and the medium containing 0.25 M glucose were similar. However, the uptake of glucose during the incubation would be expected to reduce the osmotic potential of the high-glucose medium. We found that the addition of 0.25 M mannitol to the high-glucose medium had no effect on the partitioning of ^{14}C between amylose and amylopectin. In embryos incubated in the high-glucose medium amylose contained 36% of the ^{14}C in starch, and in embryos incubated in the high-glucose medium with 0.25 M mannitol amylose contained 35% of the ^{14}C in starch (values are means of measurements from four and two separate incubations, respectively).

Affinities of soluble and granule-bound starch synthase activities for ADPglucose. The affinity of the soluble starch synthases of the developing pea embryo for ADPglucose was measured on desalted, soluble extracts. The soluble activity of the embryo is contributed by at least three different isoforms of starch synthase (Craig

et al. 1998): our measurements are representative of the soluble activity as a whole rather than of a specific isoform. We consider it highly unlikely that any enzymes other than starch synthase would have contributed to metabolism of ADPglucose in our assays. A desalted extract would not contain appropriate substrates for either sucrose synthase or ADPglucose pyrophosphorylase, the only enzymes in pea embryos other than starch synthase which can potentially metabolise ADPglucose. A value for $S_{0.5[ADPglucose]}$ of 0.13 ± 0.02 mM (mean ± SE) was estimated from Hanes plots of measurements made on five separate extracts of embryos.

Estimates of the affinity of granule-bound starch synthase for ADPglucose were made on isolated starch granules in the presence of concentrations of maltotriose known to be saturating for amylose synthesis (Denyer et al. 1996, 1999). At least 80% of the granule-bound activity of starch granules from developing pea embryos is attributable to GBSSI, the isoform responsible for amylose synthesis. The synthesis of amylose by this isoform in isolated granules requires the inclusion of malto-oligosaccharides in the assay. In the absence of these compounds the enzyme elongates chains within the amylopectin fraction (Denyer et al. 1996). A value of $S_{0.5[ADPglucose]}$ of 1.37 ± 0.2 mM (mean ± SE) was estimated from Hanes plots of measurements made on six separately prepared batches of starch.

Discussion

Our results provide strong support for the idea that the changes in the rate of starch synthesis can affect the relative rates of synthesis of amylose and amylopectin via changes in the concentration of ADPglucose. First, we have demonstrated a correlation between ADPglucose concentration and the relative rates of amylose and amylopectin synthesis in two independent ways. Developing embryos of mutant lines with reduced rates of starch synthesis have lower amounts of ADPglucose and lower contents of amylose in their starches than developing wild-type embryos. Detached wild-type embryos maintain ADPglucose contents and relative rates of amylose and amylopectin synthesis similar to those in vivo when incubated with high concentrations of glucose, but at very low concentrations of glucose both the ADPglucose content and the rate of amylose synthesis relative to that of amylopectin are substantially lowered. These effects are independent of the length and osmotic potential of the incubation. Although neither the results with mutants nor those with detached embryos constitutes proof of a causal connection between ADPglucose contents and the relative rates of amylose and amylopectin synthesis, taken together they suggest strongly that such a connection exists.

Second, differences in the affinities for ADPglucose between the granule-bound, amylose-synthesising starch synthase activity and the soluble, amylopectin-synthesising activity offer an explanation for a causal relationship between ADPglucose contents and relative rates of amylose and amylopectin synthesis. Based on the ADPglucose contents (nmol per g fresh weight) of attached embryos (from measurements on the "winter" embryos in Table 1: means ± SE of 66 ± 5, 14 ± 2, 17 ± 2, 33 ± 6 for wild-type, *rb*, *rug3* and *rug4* respectively) and the assumption that in wild-type and mutant pea embryos the ADPglucose is confined to a compartment (plastid stroma) which occupies 7% of the volume of the cell (Kruger and ap Rees 1983) we estimate that the concentration of ADPglucose in the plastid is about 0.9 mM in wild-type embryos and about 0.2–0.4 mM in mutant embryos. Based on the $S_{0.5}$ values of the starch synthases, a reduction in ADPglucose concentration from 0.9 to 0.2 mM would be expected to reduce flux through the soluble starch synthases by a factor of 1.5 and through the granule-bound starch synthase by a factor of 2.6. Thus if the rate of amylose synthesis were 27% of that of amylopectin synthesis at 0.9 mM ADPglucose, the rate at 0.2 mM ADPglucose would be approximately 18%. This figure is strikingly similar to our measurements of the amylose content of the starch of mutant embryos and the relative rate of amylose synthesis of detached wild-type embryos at low glucose concentrations.

It seems likely that the relationship between ADPglucose concentration and the relative rates of amylose and amylopectin synthesis will also apply to other starch-storing organs. Previous measurements of the affinity of granule-bound starch synthases for ADPglucose are not directly comparable with ours because they were made in the absence of malto-oligosaccharides which may be required for amylose synthesis. However, there is a broad agreement that the affinity of soluble starch synthases for ADPglucose is higher than that of the granule-bound enzyme. Estimates of K_m for soluble isoforms (either in crude extracts or purified) range from 0.06 mM to 0.6 mM (e.g. wheat endosperm, Jenner et al. 1995; pea embryo, Denyer and Smith 1992; maize endosperm, Ozbun et al. 1971, Macdonald and Preiss 1985; *Chlamydomonas*, Ball et al. 1991) whereas estimates for granule-bound activity are generally 1–3 mM (e.g. maize endosperm, Nelson et al. 1978; *Amaranthus* perisperm, Vos-Scheperkeuter et al. 1986; *Chlamydomonas*, Van de Wal et al. 1998). If the concentrations of ADPglucose available to starch synthases in other starch-storing organs are similar to those in pea embryo, changes in the concentration would be expected to alter the ratio of amylose to amylopectin in the starch.

Our estimates suggest that GBSSI in wild-type pea embryos is far from saturated with respect to ADPglucose, and it seems likely that this is the case in storage organs generally. An increase in the concentration of ADPglucose in the plastid might therefore be expected to increase in flux through GBSSI and hence increase the amylose content of the starch. Recent results from transgenic potatoes with altered expression of the plastidial adenylate transporter appear to support this prediction. Tjaden et al. (1998) showed that both decreases and increases in the expression of the transporter bring about not only changes in the starch

content of the tubers, but also changes in the amylose content of the starch. Tubers of lines with reduced activity of the transporter had reductions of up to 50% in starch content, and, for all but one of these lines, the amylose content of the starch was lower (by 20–40%) than that of wild-type starch. Tubers of lines with increased activity of the transporter had increases of up to 35% in starch content, and the amylose content of the starch was up to 40% higher than that of wild-type starch. The authors suggested that changes in the capacity of the ATP transporter affect the rate of synthesis and hence the concentration of ADPglucose within the plastid, thus altering the relative rates of amylose and amylopectin synthesis.

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