Interaction with amylopectin influences the ability of granule-bound starch synthase I to elongate malto-oligosaccharides

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This paper examines the properties in soluble form of two isoforms of starch synthase. One of these, granule-bound starch synthase I (GBSSI), is responsible for the synthesis of amylose inside the amylopectin matrix of the starch granule *in vivo*. The other, starch synthase II (SSII), is involved in amylopectin synthesis. Both isoforms can use amylopectin and malto-oligosaccharide as substrates *in vitro*. As well as acting as a substrate for GBSSI, amylopectin acts as an effector of this isoform, increasing the rate at which it elongates malto-oligosaccharides and promoting a processive rather than distributive mode of elongation of these compounds. The affinity of GBSSI for amylopectin as an effector is greater than its affinity for amylopectin as a substrate. The rate and mode of elongation of malto-oligosaccharides by SSII are not influenced by amylopectin. These results suggest that specific interaction with amylopectin in the matrix of the starch granule is a unique property of GBSSI and is critical in determining the nature of its products.

Key words: amylose, malto-oligosaccharide, starch granule.

INTRODUCTION

The aim of this work was to investigate the properties of two isoforms of starch synthase, granule-bound starch synthase I (GBSSI) and starch synthase II (SSII) in soluble form, in the absence of the starch granule. GBSSI and SSII are members of two distinct classes of starch synthase which are present in a wide range of starch synthesizing plant and algal cells. These two classes have different roles *in vivo*. GBSSI is exclusively responsible for the synthesis of amylose, the smaller, relatively unbranched component of the starch granule. SSII is one of several classes of isoform of starch synthase involved in the synthesis of the larger, highly branched polymer, amylopectin [1,2].

Comparison of GBSSI and amylopectin-synthesizing isoforms of starch synthase such as SSII has so far revealed two major differences. First, the amylopectin-synthesizing isoforms have N-terminal domains which GBSSI lacks (SSI [3,4]; SSII [5,6]; SSIII [7–9]). The amino acid sequences of the N-terminal domains are not highly conserved between orthologues from different plant species, and their function is unknown. However, for SSI and SSII, we know that the N-terminal domain is not required for amylopectin elongation *in vitro*. N-terminally truncated forms of pea SSII and maize SSI expressed in *Escherichia coli* have been shown to be active and able to use amylopectin as a substrate [10,11].

Secondly, GBSSI and SSII within starch granules differ in their ability to use malto-oligosaccharides as substrates. This was revealed in experiments with purified starch granules isolated from developing pea embryos and potato tubers [12,13]. When granules containing GBSSI and SSII were supplied with ADP-glucose and small malto-oligosaccharides, GBSSI displayed a high affinity for the malto-oligosaccharides and elongated these in a processive manner to form long glucan chains within the granule matrix. In contrast, SSII displayed a lower affinity for malto-oligosaccharides than GBSSI and, rather than elongating processively, it added a single glucose residue from ADP-glucose at each enzyme–glucan encounter. We proposed from this work that the high affinity for malto-oligosaccharides and the processive action of GBSSI may be major differences between it and the amylopectin-synthesizing isoforms in general, and that this may account for the inability of the latter to synthesize amylose.

In starch granules isolated from *Chlamydomonas reinhardtii*, amylose can be synthesized both by elongation of malto-oligosaccharides by GBSSI and by the elongation of amylopectin chains and their subsequent cleavage [14]. This led to the proposal that the synthesis of amylose via cleavage of amylopectin chains may also occur in higher plants [15]. However, no evidence of amylose synthesis by this mechanism in the starch-storing organs of higher plants has yet been presented. In pulse–chase experiments using starch from developing pea embryos, we found no evidence for movement of ^{14}C-labelled glucan chains from the amylopectin to the amylose fraction [13]. Thus, it is possible that this alternative means of generating amylose is unique to *Chlamydomonas* and we will not consider it further in this paper.

Our previous experiments (described above) do not reveal whether the unique ability of GBSSI in isolated starch granules to elongate malto-oligosaccharides in a processive manner is directly due to intrinsic differences in catalytic properties between GBSSI and SSII. This ability could also be due to indirect effects of the granule matrix on the properties of the isoforms. For example, GBSSI may be located in a different region of the granule from the other granule-bound isoforms of starch synthase, such that it is more accessible to malto-oligosaccharides. Alternatively, the mechanism of action of GBSSI, but not SSII and the other amylopectin-synthesizing isoforms, on malto-oligosaccharides may be influenced by interactions with the matrix of the starch granule. GBSSI protein within the plastid is almost entirely granule-bound, indicating that it interacts strongly with the granule matrix. The interactions of the amylopectin-synthesizing isoforms with the starch granule are probably

Abbreviations used: GBSSI, granule-bound starch synthase I; HPAEC, high-performance anion-exchange chromatography; SSII, starch synthase II.

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weaker than those of GBSSI, since these enzymes are at least partly soluble *in vivo* [1].

To discover the importance of the granule matrix in determining the properties we have defined previously for granule-bound isoforms of starch synthase, we have examined the mode of action on malto-oligosaccharides of GBSSI and SSII in soluble form. Experiments have been done in the presence and absence of amylopectin (which constitutes the matrix of the starch granule) to discover whether this glucan directly influences the mode of action of either isoform.

EXPERIMENTAL

Bacterial strain

All constructs were expressed in *E. coli* RH98, a glycogen synthase (glgA)-deficient mutant which was a gift from Dr. R. Hengge-Aronis (Department of Biology, University of Konstanz, Germany). The T7 RNA polymerase gene under the control of the isopropyl-1-thio-β-D-galactopyranoside-inducible lacUV5 promoter [16] was introduced into RH98 cells using a DE3 lysogenization kit (Novagen Inc., Madison, WI, U.S.A.) in accordance with the manufacturer's instructions.

Construction of expression plasmids

The plasmid for expression of SSII from pea (pMUT13) was constructed as described in [10]. The plasmid for expression of GBSSI from potato was constructed as follows. An ATG initiation codon was introduced at the start of the DNA encoding mature potato GBSSI (EMBL accession number: X58453) using PCR mutagenesis. The 5′ mutant oligonucleotide was: 5′ TTT GTG GAA AGG CCA TGG ACT TGA TCT TTG 3′, which also introduced an *Nco*I site at the 5′-end of the DNA encoding the mature protein. The plasmid was constructed by ligating the 1.8 kb *Nco*I–*Bam*HI fragment encompassing the cDNA encoding the mature protein, between the *Nco*I and the *Bam*HI sites of the expression vector pET3-d [17], producing pMUT6.

Expression of starch synthases in *E. coli*

The expression plasmids were transformed into competent *E. coli* RH98(DE3). Single colonies were used to inoculate 10 ml of Luria–Broth, pH 7.0 (10 g of bactotryptone, 5 g of bactoyeast extract and 10 g of NaCl per litre), containing 100 μl·ml^{-1} ampicillin, 0.66 M sorbitol and 2.5 mM betaine. After incubation at 30 °C overnight with shaking (250 rev./min), 1.25 ml were subcultured into 50 ml of the same medium and grown at 30 °C with shaking (250 rev./min) until D_{600} reached 0.6–1.0. Protein expression was induced by the addition of isopropyl-1-thio-β-D-galactopyranoside to a final concentration of 1 mM. Incubation was continued for 3 h at 20 or 30 °C with shaking at 350 rev./min. Cells were harvested by centrifugation (10000 ***g*** for 10 min) and the pellet was resuspended in 1 ml of extraction buffer [100 mM Mops, pH 7.2, 5 mM $MgCl_2$, 5 % (v/v) glycerol, 2 mM dithiothreitol and 0.1 % (w/v) BSA]. Cells were either used immediately after harvest or frozen in liquid nitrogen and stored for no longer than two weeks at −80 °C. Cells were disrupted using a French pressure cell and a soluble extract was prepared by centrifugation at 15000 ***g*** at 4 °C for 10 min. We found no significant differences between freshly prepared and frozen extracts for activities of SSII and GBSSI (results not shown).

For both constructs, the starch synthase activity per ml of extract varied between cultures. Replicate cultures grown at the same time from separate colonies of *E. coli* showed less variation in activity per ml of extract than cultures grown at different times. Where mean values for activity of starch synthase are presented, these were obtained from measurements on cultures grown at the same time from separate colonies.

Assay of starch synthase activity

Assays contained (in a final volume of 100 μl) 100 mM Bicine (pH 8.5), 25 mM potassium acetate, 10 mM dithiothreitol, 5 mM EDTA, 1 mM ADP[U-^{14}C]glucose (Amersham International, Bucks., U.K.) at 2.3 GBq·mol^{-1}, 100 mM or 0 mM maltotriose, 0 M or 0.5 M sodium citrate, 0–50 mg·ml^{-1} potato amylopectin, oyster glycogen or rabbit liver glycogen (Sigma, Poole, Dorset, U.K.) and 10 μl of *E. coli* extract. Assays were incubated at 25 °C for 10–30 min. Controls were terminated immediately after the addition of extract to the assay. All assays and controls were duplicated. Assays were terminated by heating to 90–100 °C for 2 min. In experiments where assays contained a range of concentrations of amylopectin, the amylopectin content was equalized after the assays had been terminated. Assays were processed by one of the following methods.

Methanol/KCl method

Aqueous methanol (3 ml, 750 ml·l^{-1}) containing 10 g·l^{-1} KCl (methanol/KCl) was added and assays were processed as described in [13]. This method was used to measure the products of elongation of amylopectin.

Resin method

Assays containing more than 5 mg·ml^{-1} amylopectin or glycogen were incubated at 37 °C for 3 h with amyloglucosidase and α-amylase as described in [13], before processing on columns of Dowex 1-X8. Assays containing less than 5 mg·ml^{-1} amylopectin or glycogen were processed on Dowex columns without prior digestion. This method was used to measure the products of elongation of both amylopectin and malto-oligosaccharides.

To determine whether our assays gave reliable estimates of the activities of GBSSI and SSII, incubations were performed using a range of extract concentrations and incubation times. For SSII, the activity was linearly dependent upon both the amount of extract added to the assays and on the incubation times. For GBSSI, the activity between 0 and 10 min was lower than that between 10 and 30 min, and with very dilute extracts the activity was underestimated (results not shown). The non-linearities in assays of GBSSI activity introduced small errors in estimates of GBSSI activity of no more than 10–15 % and did not affect the conclusions drawn from any of the experiments reported in this paper. To minimize these errors, in some experiments (e.g. Figure 3), activities were calculated from the difference between the product formed at 10 min and that at 20 min (or 20 and 30 min) rather than from the difference between the product formed at 0 min and that at 10, 15 or 20 min.

Assay of amylopectin-degrading enzymes

Extracts of *E. coli* were assayed for starch synthase activity as described above. The assays included amylopectin and were processed by the methanol/KCl method. The precipitated amylopectin was re-dissolved in 100 μl of an incubation mixture containing 100 mM Bicine (pH 8.5), 25 mM potassium acetate, 10 mM dithiothreitol, 5 mM EDTA and either 10 μl of *E. coli* extract or 10 μl of water. After incubation at 25 °C for 15 min, assays were terminated and processed by the methanol/KCl method as above.

Preparation of samples for high-performance anion-exchange chromatography (HPAEC)

Samples of ^{14}C-labelled products of starch synthases were prepared using the resin method as described above, except that the incubations were for 1 h, the specific activity of ADP-glucose was eight times higher and the Dowex columns were eluted with two successive additions of 20 μl of water.

Analysis by HPAEC

Analysis by HPAEC was as described in [13] for analysis of soluble products.

SDS/PAGE and immunoblotting

SDS/PAGE and immunoblotting were carried out as described in [18].

RESULTS

Expression of isoforms of starch synthase in *E. coli*

In preliminary experiments to establish appropriate experimental systems, genes encoding the mature GBSSI and SSII proteins from both pea [5,10] and potato [5,19] were expressed in a mutant strain of *E. coli*, RH98 (DE3), which lacks glycogen synthase activity. Potato GBSSI and pea SSII were both expressed as proteins of expected mass in a soluble and active form. However, pea GBSSI displayed almost no activity and potato SSII was expressed as both full-length and extensively truncated forms. Analysis of the properties of the isoforms was therefore conducted only on potato GBSSI and pea SSII. Conditions for induction and growth of the *E. coli* were optimized for maximum expression of starch synthases in active and soluble form. Proteins of the expected masses were routinely detected by immunoblotting (Figure 1). No proteins of these masses were detected in *E. coli* transformed with the vector (PET3-d) alone.

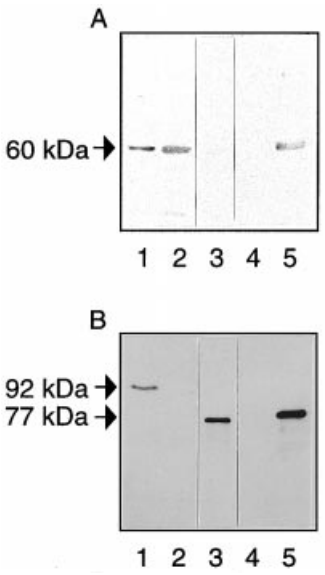

Figure 1 Expression of starch synthases in *E. coli*

Cells were suspended in extraction buffer without BSA and disrupted as described in the Experimental section. The soluble extract was diluted 1:40 in gel sample buffer, heated to 90 °C for 2 min and loaded onto 6% SDS/polyacrylamide gels. The gels were blotted on to nitrocellulose and the blots developed with antiserum to (**A**) pea GBSSI [22] (dilution: 1/2500) or (**B**) a truncated form of pea SSII [10] (dilution: 1/10000). Lanes contained: 1, proteins from potato starch; 2, *E. coli* cells transformed with pMUT6 and expressing mature potato GBSSI; 3, *E. coli* cells transformed with pMUT13 and expressing mature pea SSII; 4, *E. coli* cells transformed with the vector pET-3 alone; and 5, proteins from pea starch. Molecular masses are indicated.

Starch synthase activity was detected in extracts of *E. coli* expressing GBSSI and SSII. Extracts of *E. coli* transformed with the vector only had no detectable activity. The concentration of potato GBSSI protein in extracts of *E. coli* was estimated by gel-densitometry of Coomassie Brilliant Blue-stained SDS/polyacrylamide gels using BSA as a standard. From these measurements, and estimates of the activity of GBSSI (in assays containing 5 mg·ml^{-1} amylopectin and no malto-oligosaccharide), the specific activity was estimated to be 0.16 μmol·min^{-1}·mg^{-1} of protein. SSII was expressed less strongly than potato GBSSI and could not be seen clearly on Brilliant Blue-stained SDS/polyacrylamide gels of extracts of *E. coli* (results not shown). This prevented estimation of its specific activity by this method.

To determine whether there were starch-degrading enzymes in the extracts of *E. coli* which might interfere with measurements of starch synthase activity, samples of ^{14}C-labelled amylopectin produced by GBSSI or SSII were incubated with crude extracts of *E. coli* or with water. The amount of labelled amylopectin recovered after incubation with extract was 98.6 $\pm$ 16.3 % (mean $\pm$ S.D. of values from four experiments, each with a separate culture of *E. coli*) of that recovered after incubation with water. This shows that the crude extract at the concentrations used in our assays contains no enzymes that can degrade amylopectin to an extent likely to generate artifactually low values in starch synthase assays. Crude, soluble extracts were therefore used as sources of starch synthase activity in subsequent experiments.

Activities of GBSSI and SSII with amylopectin

The activity of both enzymes increased with increasing amylopectin concentration (Table 1). Over the range 5–50 mg·ml^{-1} amylopectin, the proportional increase in the activity of GBSSI was greater than that for SSII. This suggests that the affinity of GBSSI for amylopectin was lower than that of SSII. For SSII, the Michaelis constant (K_m value) for amylopectin from measurements over a range of amylopectin concentrations (results not shown) was calculated to be 4.6 $\pm$ 1.0 mg·ml^{-1} (mean $\pm$ S.D. of values from four experiments, each on a separate culture of *E. coli*). Similar calculations for GBSSI gave a value of 31.2 $\pm$ 11.18 mg·ml^{-1} (mean $\pm$ S.D. values from four experiments, each on a separate culture of *E. coli*; see Figure 3, open circles). However, use of the Michaelis–Menten equation to calculate a K_m value for amylopectin may not be appropriate as, at least under some conditions, amylopectin can act as an effector as well as a substrate of GBSSI (see below). In addition, changes in the physical properties of solutions of amylopectin with increasing concentration can lead to activation of GBSSI [20].

Activities of GBSSI and SSII with maltotriose

Both GBSSI and SSII elongated maltotriose when this was supplied at concentrations previously shown to be saturating for these enzymes in isolated starch granules [13]. The activity with 100 mM maltotriose was less than that with 5 or 50 mg·ml^{-1} amylopectin (Table 1). When assayed in the presence of maltotriose and 5 or 50 mg·ml^{-1} amylopectin, the activity of SSII was similar to its activity with amylopectin alone. However, for GBSSI, the activity in the presence of both substrates was greater than the sum of the activities with the two substrates inde-

Table 1 Activities of GBSSI and SSII with amylopectin and maltotriose

Crude, soluble extracts of *E. coli* expressing potato GBSSI or pea SSII were assayed for starch synthase activity as described in the Experimental section in the presence of various substrates. Results are expressed as activity per ml of extract and are the means ± S.D. of measurements on three separate cultures of *E. coli*.

Substrate	Starch synthase activity (nmol · min^{-1} · ml^{-1})	
	GBSSI	SSII
Amylopectin (5 mg · ml^{-1})	52.5 ± 7.5	558 ± 73
Amylopectin (50 mg · ml^{-1})	222 ± 38	1020 ± 150
Maltotriose (100 mM)	21.9 ± 4.2	326 ± 25
Maltotriose (100 mM) and amylopectin (5 mg · ml^{-1})	152 ± 40	705 ± 44
Maltotriose (100 mM) and amylopectin (50 mg · ml^{-1})	350 ± 64	1170 ± 240

pendently. This suggested that, for GBSSI, the elongation of one of the substrates, either amylopectin or maltotriose, was stimulated by the presence of the other. The stimulation of GBSSI activity was greatest at the lower amylopectin concentration. Further investigation of this is described below.

Amylopectin stimulates the elongation of malto-oligosaccharides by GBSSI

To determine whether the stimulation of GBSSI activity in the presence of both amylopectin and maltotriose was due to stimulation of amylopectin elongation or malto-oligosaccharide elongation, we analysed the products of GBSSI and SSII by two alternative methods designed to distinguish between these two possibilities (see the Experimental section). Amylopectin and small malto-oligosaccharides have different solubilities in solutions of alcohol. Whereas amylopectin is insoluble in methanol/KCl, malto-oligosaccharides are largely soluble. We found that the methanol/KCl treatment used in our experiments did not precipitate malto-oligosaccharides of less than 25 glucose residues (results not shown). Thus the resin method measures the incorporation of labelled glucose into both amylopectin and malto-oligosaccharides, whereas the methanol/KCl method reveals the incorporation of labelled glucose into amylopectin but not into small malto-oligosaccharides.

The two methods gave similar results when GBSSI and SSII were incubated with amylopectin alone (Figure 2). This showed that there was no degradation of amylopectin during the incubations. Degradation would interfere with estimates of starch synthase activity made by the methanol/KCl method [21]. When GBSSI and SSII were incubated with maltotriose alone, products were revealed by the resin method but not by the methanol/KCl method. When GBSSI and SSII were incubated with both amylopectin and maltotriose, the resin method revealed much more product than the methanol/KCl method. Most of the activity in these incubations was, therefore, due to elongation of maltotriose, not amylopectin. For GBSSI, the rate of elongation of maltotriose was much greater in incubations containing amylopectin than in incubations containing maltotriose alone. Thus, in incubations containing both substrates, amylopectin stimulates malto-oligosaccharide synthesis by GBSSI, without itself being elongated to any significant extent.

The stimulation of maltotriose elongation by GBSSI in the presence of amylopectin could be due to an indirect effect of a high concentration of glucan in the assay or to a more specific interaction between GBSSI and amylopectin. To investigate this, we compared the stimulatory effect of amylopectin with that of the same concentration (5 mg · ml^{-1}) of glycogen. The activity of GBSSI on maltotriose was stimulated 6.34 ± 0.10-fold by addition

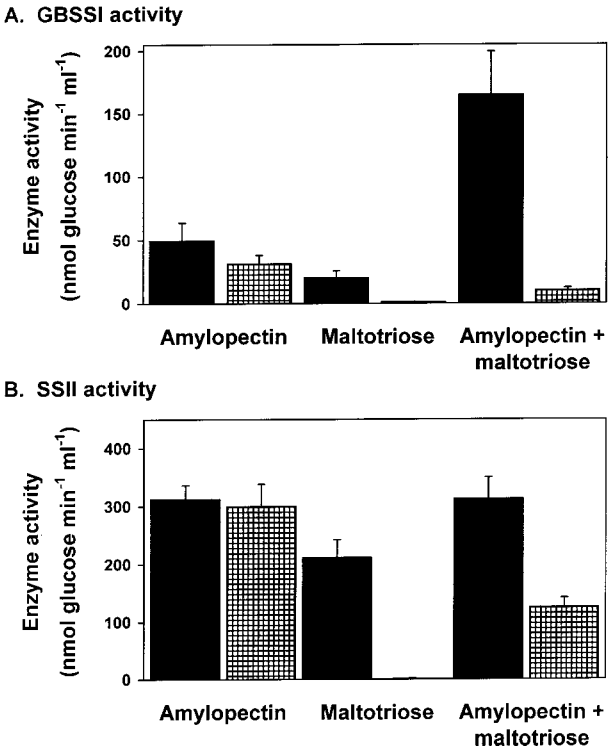

Figure 2 Elongation of maltotriose by GBSSI is stimulated by amylopectin

Crude, soluble extracts of *E. coli* transformed with pMUT6 (**A**, GBSSI) or pMUT13 (**B**, SSII) were prepared and assayed for starch synthase activity as described in the Experimental section. Incubations contained 5 mg · ml^{-1} amylopectin, 100 mM maltotriose, or both of these, and duplicate incubations were processed by the resin (black bars) and the methanol/KCl (hatched bars) methods. Values are means ± S.D. of measurements of three separate cultures of *E. coli*.

of amylopectin and 1.49 ± 0.22-fold by addition of glycogen (means ± S.D. of values from three experiments, on three separate cultures of *E. coli*). Thus, the stimulatory effect of amylopectin is far greater than that of glycogen. This may indicate that there is a specific interaction between GBSSI and amylopectin which increases the ability of the enzyme to elongate maltotriose. However, the physical properties of amylopectin-containing solutions are probably different from those of solutions containing glycogen, and these could be responsible for the observed effects on GBSSI activity.

To determine the concentration of amylopectin required to stimulate malto-oligosaccharide synthesis, we incubated extracts

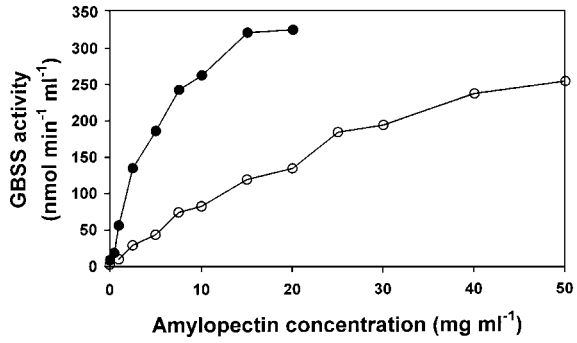

Figure 3 Affinity of GBSSI for amylopectin

Crude, soluble extracts of *E. coli* transformed with pMUT6 (GBSSI) were prepared and assayed for starch synthase activity as described in the Experimental section in the presence (●) or absence (○) of 50 mM maltotriose. Incubations containing various concentrations of amylopectin were performed in duplicate and were terminated after 10, 20 or 30 min and then processed by the resin method. The incorporation of label was linear over 10–30 min. GBSSI activity was calculated as the mean of values for 10–20 and 20–30 min. Results from a typical experiment are shown. Data were analysed using Hanes plots and the results of these analyses are given in the text.

containing GBSSI and ADP[^{14}C]glucose with 0 or 50 mM maltotriose and various concentrations of amylopectin and processed the assays by the resin method (Figure 3). Processing of replicate assays by the methanol/KCl method confirmed that essentially all of the product in assays containing both maltotriose and amylopectin was elongated maltotriose. The elongation of maltotriose was stimulated by increasing concentrations of amylopectin (Figure 3, closed circles). The concentration of amylopectin required to saturate this stimulatory effect was considerably lower than that required for maximal activity in assays containing only amylopectin (Figure 3, open circles). The concentration of amylopectin which gave half the maximum rate of maltotriose elongation was 4.8 ± 1.0 mg·ml^{-1} (mean ± S.D. of estimates from five separate cultures of *E. coli*, calculated using Hanes plots). In assays containing amylopectin only, the concentration of amylopectin which gave half the maximum rate of elongation was 31.2 mg·ml^{-1} (see above).

It was not possible to measure the affinities of GBSSI and SSII for maltotriose because for both enzymes the activities were low and increased linearly with increasing concentrations of maltotriose up to 1 M (results not shown). To determine the affinity of GBSSI for maltotriose in the presence of amylopectin, we measured the activity of GBSSI in the presence of 5 mg·ml^{-1} amylopectin and a range of concentrations of maltotriose. Assays were processed by both the resin and methanol/KCl methods, and results from the latter method were subtracted from those from the former method to determine the rate of elongation of maltotriose. GBSSI activity was substantially saturated at 100 mM maltotriose in the presence of amylopectin, and the estimated K_m was 15.3 ± 5.4 mM (mean ± S.D. of values from three experiments, on two separate cultures of *E. coli*, calculated using Hanes plots).

Mode of elongation of malto-oligosaccharides by GBSSI and SSII

To discover the mode of elongation of malto-oligosaccharides by GBSSI and SSII, we examined the nature of their products in incubations containing ADP[^{14}C]glucose and high concentrations (100 mM) of maltotriose. If a single glucose residue was added to maltotriose before enzyme and product dissociated (distributive

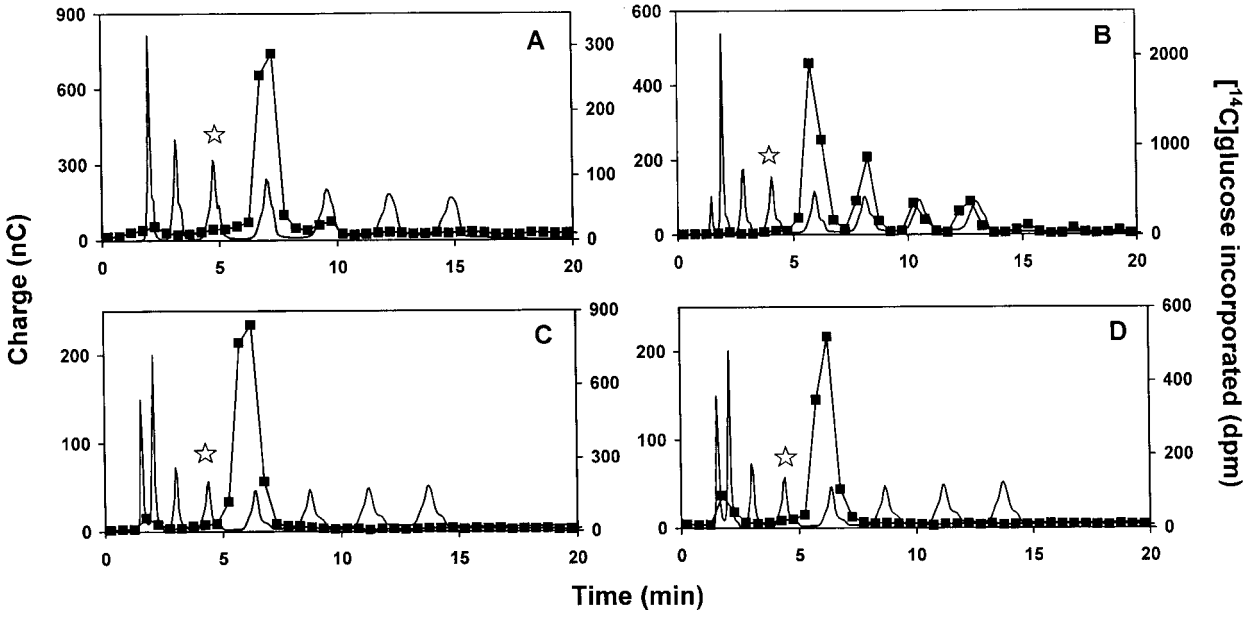

Figure 4 Mode of elongation of maltotriose

Crude, soluble extracts of *E. coli* transformed with pMUT6 (GBSSI: **A** and **B**) or pMUT13 (SSII: **C** and **D**) were prepared and assayed for starch synthase activity as described in the Experimental section. Incubations contained 100 mM maltotriose alone (**A** and **C**) or 100 mM maltotriose and 2 mg·ml^{-1} amylopectin (**B** and **D**) and were processed by the resin method. Labelled products were analysed by HPAEC on a CarboPac PA100 column. The elution of ^{14}C was monitored by liquid-scintillation counting (■) and the elution of standards (glucose, maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose and maltoheptaose) was monitored with a pulsed amperometric detector (solid line). The position of elution of the maltotriose standard is indicated by a star. Results from typical experiments are shown.

mode of action) then maltotetraose would be the expected product. However, if the maltotriose was elongated processively, both maltotetraose and products larger than maltotetraose would be expected [13]. For both enzymes, the product was largely or entirely maltotetraose (Figures 4A and 4C), indicating a predominantly distributive mode of elongation. In three separate experiments with GBSSI, maltotetraose accounted for $88 \pm 9\%$ (mean $\pm$ S.D.), and in two experiments with SSII, maltotetraose accounted for 96% and 84% respectively of the total amount of radioactive product recovered from the column. The radioactive products recovered from the column in these experiments accounted for $96 \pm 5.5\%$ (mean $\pm$ S.D.) of the radioactivity initially applied to the column, showing that there were no major losses of products during chromatography.

When amylopectin ($2\ mg \cdot ml^{-1}$) was included in the incubations, as well as maltotriose, products larger than maltotetraose were synthesized by GBSSI but not by SSII (Figures 4B and 4D). The synthesis of products longer than maltotetraose in the presence of amylopectin was observed in incubations containing a range of different activities of GBSSI (results not shown). The nature of the products of GBSSI was thus dependent upon the presence or absence of amylopectin and not upon the activity of GBSSI in the incubation. In three separate experiments with GBSSI, $45 \pm 5\%$ (mean $\pm$ S.D.) of the total products were longer than maltotetraose. In experiments with SSII, products longer than maltotriose accounted for less than 1% of the total products. The radioactive products recovered from the column in these experiments accounted for $82 \pm 8\%$ (mean $\pm$ S.D.) of the radioactivity initially applied to the column. These results showed that interactions between amylopectin and GBSSI influenced the mode of action of the enzyme, promoting a processive mode of elongation of maltotriose by GBSSI.

DISCUSSION

Our results show that the properties of GBSSI in a soluble state differ considerably from those we reported earlier [12,13] for the enzyme within isolated starch granules. First, whereas GBSSI within granules has a high affinity for malto-oligosaccharides (the $S_{0.5}$ value, which is the concentration of maltotriose at which the rate of amylose synthesis was half its limiting rate, was 0.1 mM), the soluble enzyme has a very low affinity (not saturated at 1 M maltotriose). Secondly, granule-bound GBSSI elongated malto-oligosaccharides in a processive manner, adding more than one glucose unit per enzyme–glucan encounter, whereas soluble GBSSI acts in a largely distributive manner, adding only one glucose unit per enzyme–glucan encounter.

Amylopectin in a soluble state acts as both a substrate and an effector for GBSSI. As an effector, it stimulates the rate of elongation of malto-oligosaccharide and it increases the affinity of the enzyme for this substrate. It also alters the mode of action of the enzyme on malto-oligosaccharide, changing it from a predominantly distributive to a processive enzyme. The mechanism by which GBSSI activity is altered in the presence of amylopectin is not clear. We postulate that amylopectin may induce a conformational change in GBSSI such that the affinity of GBSSI for malto-oligosaccharides is increased, the rate of dissociation of GBSSI from the newly elongated malto-oligosaccharide is decreased and the mode of action of the enzyme on this substrate thus becomes increasingly processive. There is a distinct difference between the low concentration of amylopectin required to activate the elongation of malto-oligosaccharides by GBSSI and the much higher concentrations of amylopectin required to saturate the enzyme when amylopectin is the sole substrate. This suggests that amylopectin interacts with GBSSI in two distinct ways. The fact that amylopectin is an effector of GBSSI with respect to its activity on malto-oligosaccharide raises the possibility that it acts as both a substrate and an effector in assays which contain amylopectin alone. We are investigating this further (see [20]).

Soluble amylopectin confers on GBSSI properties with respect to malto-oligosaccharides that are similar to those of the enzyme within the granule. At present, we do not know whether this is due to a specific interaction between the enzyme and the amylopectin molecule or whether the physical environment created by amylopectin in solution mimics the environment within the starch granule. However, there is some evidence to suggest that GBSSI does interact with the amylopectin component of the starch granule. Studies of amylose-free mutants of several species which have inactive forms of GBSSI [22,23] have shown that GBSSI is still predominantly granule-bound, even though the granule lacks amylose. This suggests that the binding of wild-type GBSSI to starch granules may be largely or entirely due to an interaction with the amylopectin component rather than the amylose component of the granule.

The SSII isoform of starch synthase differs from GBSSI in that amylopectin acts only as a substrate, and not as an effector, for the enzyme. With respect to malto-oligosaccharides, the properties of soluble SSII are similar to those of granule-bound SSII reported earlier [12,13]. The activity of the enzyme is not stimulated by amylopectin and its distributive mode of action on malto-oligosaccharides is not altered by the addition of amylopectin. Further work is in progress to define the structural features of GBSSI and SSII responsible for their differing modes of elongation of malto-oligosaccharides and their interaction with amylopectin (see [20]).

Previously [13], we suggested that the role of GBSSI in amylose biosynthesis might be explained by its high affinity for malto-oligosaccharides and unique ability to elongate these substrates processively, together with the ability of the granule matrix to trap longer malto-oligosaccharide chains. The work in this paper describes intrinsic differences between GBSSI and SSII in their interactions with malto-oligosaccharides, which are consistent with our proposal. The effect of amylopectin on the properties of GBSSI in a soluble state suggests that interactions between GBSSI and amylopectin within the matrix of the granule may be essential to allow processive elongation of malto-oligosaccharides by GBSSI, and thus to allow amylose biosynthesis. The processive mode of elongation may also explain the contribution of GBSSI to the synthesis of very long chains in the amylopectin fraction of starch granules isolated from higher plants [3,12] and *Chlamydomonas* [14]. Overall, we suggest that the ability to interact with the amylopectin matrix and thus attain a processive mode of action may be the primary feature that distinguishes GBSSI from isoforms of starch synthase which cannot synthesize amylose.

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