STARCH SYNTHASES

I. INTRODUCTION

After the synthesis of the glucosyl donor by the ADPglucose pyrophosphorylase (ADPGlc PPase), the next reaction in the starch biosynthetic pathway involves the transfer of the glucosyl moiety of the sugar nucleotide to a maltosaccharide, glycogen, or starch, forming a new α-1,4-glucosidic linkage. In this step, there are some differences between the bacterial and plant systems. In bacteria such as *Escherichia coli,* only one glycogen synthase, encoded by one glycogen synthase gene, has been found (Kumar *et al.,* 1986). Conversely, in every plant tissue studied, more than one starch synthase has been identified (Preiss, 1982a,b, 1991; Preiss and Romeo, 1989; Sivak and Preiss, 1994; Preiss and Sivak, 1996) and they are encoded by more than just one gene. Some starch synthases are bound to the starch and can only be solubilized by α-amylase digestion of the granule, whereas others, designated as soluble starch synthases (SSS), are found in the soluble portion of the extract.

The biochemical and molecular biology characterization of the multiple forms of starch lags behind that of the other enzymes in starch biosynthesis, a problem that can be attributed to the instability of some of the isoforms.

II. SOLUBLE STARCH SYNTHASES

Work with a variety of plant systems has shown that multiple forms of SSS are present. Studies on barley endosperm, pea seeds, wheat endosperm, sorghum seeds, teosinte seeds, spinach leaf, maize endosperm, potato tuber, and rice seed extracts have indicated the presence of at least two major forms of SSS (reviewed in Preiss and Levi, 1980; Preiss, 1988; Preiss and Sivak, 1996), designated as types I and II. In maize leaf (Dang and Boyer, 1988) and castor bean endosperm (Goldner and Beevers, 1989), only one form of starch synthase was found, but since no extensive purification was attempted, the possibility remained that existing multiforms were not separated. Indeed, Downton and Hawker (1973) did find two forms of starch synthase in maize leaf, and thus the issue of the number of forms

in maize leaf remains unresolved. It is important to note that Downton and Hawker (1973) found much greater activity in their extracts than that reported by Dang and Boyer (1988), and the possibility of incomplete extraction by the latter remains.

In maize kernels, soluble starch synthase I (SSSI) elutes from anion exchange columns at lower salt concentrations than soluble starch synthase II (SSSII). Although SSSI has been partly purified from maize kernels, SSSII is more unstable and has been more difficult to purify. In our laboratory, the use of several purification steps has resulted in enzymatic fractions with relatively high specific activities (2.6 and 4.2 μmol glucose incorporated per minute per mg protein for SSSI and SSSII, respectively), and these enzymatic fractions were free from amylases and branching enzyme. The apparent affinity for ADPglucose, measured by the K_m, is similar for the two forms (Table I). The maximal velocity of the type I enzyme is greater with rabbit liver glycogen than with amylopectin, and the type II enzyme is less active with glycogen than with amylopectin. Citrate stimulation of the primed reaction is greater for type I than for type II. Both forms can use the oligosaccharides maltose and maltotriose as primers when present at high concentrations. Starch synthase I seemed to have more activity than SSSII with these acceptors (Macdonald and Preiss, 1985).

The lower activity for SSSI with amylopectin as a primer, as compared with glycogen, suggests that SSSI may have a higher preference for the short exterior chains (A-chains) that are more prevalent in glycogen than in amylopectin. The reverse may be true for SSSII, where SSSII may have preference for the longer A-chains and B-chains seen in amylopectin. Dif-

TABLE I

PROPERTIES OF THE SOLUBLE STARCH SYNTHASES FROM MAIZE ENDOSPERM

Property	Starch synthase I	Starch synthase II
Molecular mass (kd)	72	95
Affinity for substrates (K_m)		
ADPGlc	0.1 mM	0.1 mM
Amylopectin	0.16 mg/ml	1.5 mg/ml
Amylopectin (with citrate present)	<1 μg/ml	0.09 mg/ml
Relative activity with different primers (amylopectin = 1)		
Amylopectin + citrate	4.4	1.8
No exogenous primer + citrate	5.8	<0.02
Rabbit liver glycogen	2.1	0.6
1.0 M maltose	1.6	1.2
0.1 M maltotriose	0.9	0.5

ferences were also noted in the apparent affinities with respect to the glucan primer. For example, the K_m for the type I enzyme for amylopectin is nine times lower than that of the type II enzyme. It is worth noting that the type I enzyme is active without added primer in the presence of 0.5 M citrate, whereas the type II enzyme is inactive. Citrate decreases the K_m of amylopectin for both types of enzymes; 160-fold for the type I enzyme and about 16-fold for the type II starch synthase with 0.5 M citrate. SSSI and SSSII enzymes also have different molecular masses based on sucrose density ultracentrifugation: SSSI of maize endosperm is about 70 kDa and SSSII is 95 kDa, and they may be immunologically distinct. Antibody prepared against maize endosperm SSSI showed little reaction with SSSII in neutralization tests. In summary, the two soluble forms of maize starch synthase seem to be distinct on the basis of their physical, kinetic, and immunologic properties, and thus are probably products of two different genes. Because of their different kinetic properties and different specificities with respect to primer activities, they may have different functions in the formation of the starch granule (Macdonald and Preiss, 1985). The properties listed in Table I for the maize isoenzymes are likely to represent the situation in other species and tissues. Both types of synthases can use the oligosaccharides—maltose and maltotriose—as primers at high concentrations. For maltose, the immediate new product was maltotriose; for maltotriose, the product was maltotetraose. Starch synthase I seemed to have more activity with the oligosaccharides than did the type II form. It is worth noting that for most (but not all) plant tissues, starch synthase I elutes from an anion exchange column at lower salt concentrations than starch synthase II.

Native type I and type II enzymes also have different molecular masses based on sucrose density ultracentrifugation (Hawker *et al.*, 1974).

It is possible to study the products formed by the action of the enzymes studied, and this is extremely useful for both starch synthase and branching enzyme. After incubation of the substrates with the enzyme, the product is isolated and debranched by the action of isoamylase (from *Pseudomonas* sp.), which hydrolyses the α-1,6 bonds but not the α-1,4 bonds, yielding relatively short linear chains. The chains obtained are characterized using high-performance anion-exchange chromatography (HPAEC, with a Dionex CarboPac PAI, 250 × 4 mm column); maltodextrin chains with a DP of up to at least 40 units can be easily separated in less than 50 minutes using as eluting solvent a linear gradient in 150 mM NaOH of 0 to 500 m*M* sodium acetate (Guan *et al.*, 1995). Other useful methods include Smith degradation, measurement of the blue value (BV), determination of carbohydrates by the phenolsulfuric method (Takeda *et al.*, 1983), and beta amylolysis limit.

In rice, three isoforms of SSS were separated by anion exchange chromatography, which, in immunoblot, reacted with antibodies raised to the rice waxy protein (Baba *et al.*, 1993). After affinity chromatography of the active fractions, amino-terminal sequences were obtained for the protein bands of 55 to 57 kD (separated by SDS–PAGE) that cross-reacted weakly with serum raised against the rice waxy protein. It is worth noting that this experimental approach does not exclude the possibility that other soluble starch isoform(s) were present that did not cross-react with the antiserum, and other results from Baba *et al.* (1993) indicate that another SSS isoform of 66 kDa is also present in seed extracts. The same authors isolated cDNA clones coding for the putative SSS from maize from an immature rice seed library in λgt 11, using as probes synthetic oligonucleotides designed on the basis of the amino-terminal amino acid sequences available. The longer insert, of about 2.5 kb, was sequenced and was shown to code for a 1878-nucleotide open reading frame. Comparison with the corresponding amino-terminal sequences led the authors to conclude that the protein is initially synthesized as a precursor carrying a long transit peptide at the amino-acid terminus and that the same gene would be expressed in both seeds and leaves.

III. STARCH SYNTHASES BOUND TO THE STARCH GRANULE

Attempts to elute or solubilize the activity had met with little success until Macdonald and Preiss (1983, 1985) incubated ground maize starch granules with α-amylase and glucoamylase. The solubilized starch synthase activity was chromatographed on an anion exchange column and two peaks of activity were obtained, with 80% of the activity residing with starch synthase I, which eluted from the DEAE-cellulose column at a lower salt concentration than the starch synthase II fraction.

The solubilized, granule-bound enzymes showed apparent affinities for ADPGlc approximately ten times higher than those measured before solubilization (0.96 m*M* for the intact granule activity; F. D. Macdonald and J. Preiss, 1985). The granule-bound enzyme is also active with UDPglucose (Macdonald and Preiss, 1983); in the presence of 0.5 M citrate and amylopectin primer, the granule-bound activity with 1 m*M* UDPGlc exhibits about 7% of the activity seen with 1 m*M* ADPGlc. If the concentration of UDPGlc is raised to 20 m*M*, then the activity is about 73% of that of ADPGlc. Upon digestion of the granule with α-amylase and glucoamylase, the UDPGlc activity essentially disappears. Thus, either the appreciable activity observed with high concentrations of UDPGlc was not solubilized (which would suggest that the UDPGlc activity is catalyzed by a different enzyme than the ADPGlc activity) or was denatured during the amylase

treatment, or the ability of the starch-bound starch synthase to use UDPGlc is dependent on the close association of the enzyme with the starch granule. Once solubilized, the granule-associated enzyme is specific for the sugar nucleotide, ADPGlc, and thus is similar to the SSS.

Other properties of the granule-solubilized starch synthases are seen in Table II and can be compared with the SSS. The granule-bound enzymes freed of starch now require a primer for activity. The granule-bound starch synthase II, in contrast to the SSSII, has a higher apparent affinity (lower K_m) for amylopectin than the granule type I enzyme. This is opposite to the soluble enzyme forms. However, as seen for the SSSI, the granule-bound starch synthase I has a higher activity with rabbit liver glycogen than with amylopectin. The starch synthases II, soluble or granule-bound, have less activity with rabbit liver glycogen than with amylopectin. The solubilized granule starch synthase I also has activity without added amylopectin in the presence of 0.5 M citrate, whereas the solubilized granule starch synthase II shows little activity in the presence of citrate and absence of primer. The solubilized granule starch synthases I and II can use oligosaccharide primers as do the soluble starch synthases; Table II shows the activities with respect to maltose and maltotriose. Other maltosaccharides can be used as primers and the products of the reaction observed with maltose, maltotriose, maltotetraose, maltopentaose, maltohexaose, and maltononaose are the maltosaccharides with an additional glucosyl unit (e.g., the product with maltopentaose was maltohexaose).

The molecular weight of the native enzymes can be determined in a number of ways, even in the presence of protein contaminants, by using sucrose density gradients or size exclusion chromatography. The major granule starch synthase, I, has a mass of 61,000, which is different than the

TABLE II

ACTIVITY OF THE STARCH GRANULE-BOUND SYNTHASES WITH ADPGlc AND UDPGlc AS SUBSTRATES: EFFECT OF GRINDING AND SOLUBILIZATION

	Starch synthase activity (nmol · min^{-1} · mg^{-1} protein)	
Enzyme fraction	1 m*M* ADPGlc	20 m*M* UDPGlc
Granules, intact	26	18.9
Granules, ground	114	40
α-amylase–treated ground starch	9.7	<0.02
DEAE-sepharose fractions		
Starch synthase I	47	<0.7
Starch synthase II	74	1.7

molecular mass of the soluble starch synthase I, which is 72,000. Both starch synthases II, soluble or granule-bound, have similar mass; approximately 93,000 (Macdonald and Preiss, 1985).

Antibody prepared against SSSI effectively neutralized SSSI activity, but has little or no effect on either the activities of granule-bound starch synthases I or II or even on SSSII. A result consistent with that obtained with the SSS antiserum is the observation that antibody prepared against the starch granule-bound proteins effectively inhibits granule-bound starch synthase I activity, but has little effect on the SSS (Macdonald and Preiss, 1985).

Classification of starch synthases is primarily based on work done on the maize endosperm enzymes, but work on other species supports, at least qualitatively, similar classifications. Thus, two broad categories of starch synthases can be defined depending on whether the enzymes are soluble or starch granule-bound. Within these categories, two classes of starch synthases I and II can be defined on the basis of their behavior on ion exchange and hydrophobic chromatography. In summary, type I elutes from DEAE-sepharose and aminobutyl-sepharose, at lower salt concentrations, have lower molecular masses, show higher activity with glycogen than with amylopectin, and show higher activities in the absence of primer with citrate or with amylopectin as primer and citrate when compared to the type II class. The two classes are also immunologically distinct. The starch granule-bound enzymes can also be distinguished immunologically from their soluble starch synthase counterparts. There are also differences in their molecular weights and in their kinetic properties. Synthesis of a starch granule has not been obtained in vitro. In vivo, synthesis occurs by deposition on the granule surface by the concerted action of starch synthases and branching enzyme. On centrifugation of a crude extract, starch synthase activity is found associated with the starch granules or in the supernatant. It is assumed that this partition is a result of differences in the structure of the isozymes and/or differences in the role they play in the synthesis of the starch components, amylose and amylopectin. Also, for storage organs such as seeds and tubers, the starch granule is formed and grows for several weeks, and it is likely that different isozymes vary in importance during this period. In maize endosperm there are at least four starch synthases: two soluble (Ozbun *et al.*, 1971) and at least two granule-bound (Macdonald and Preiss, 1985). The number of isoforms may vary with the plant species and the developmental stage, but those that have been studied more carefully seem to have a similar number of isoforms. Indeed, as in the case of the pea embryo, an isozyme of starch synthase—starch synthase II—can exist as a soluble and starch-granule bound isozyme (Edwards *et al.*, 1996). The question remains as to whether the soluble and granule-bound forms are both functional. Indeed, Mu-Forster *et al.* (1996) has reported that in maize

endosperm, more than 85% of the starch synthase I protein may be associated with the starch granule. This was determined by using an antibody prepared against the starch synthase, but no evidence was presented to indicate that the protein had starch synthase activity.

The cDNA clones that encode the two isozymes of granule-bound starch synthase of the pea embryo are optimally expressed at different times during development (Dry *et al.*, 1992); although isozyme II is expressed in every organ, isozyme I is not expressed in roots, stipules, or flowers (Dry *et al.*, 1992).

It is worth noting that the understanding of the starch synthases lags behind that of the ADPGlc PPase and the branching enzymes. To cover that ground, it will be necessary to achieve expression of the plant enzymes in *E. coli* so that studies of structure–function relationships can be facilitated.

IV. ISOLATION OF THE WAXY PROTEIN STRUCTURAL GENE

Amylose content determines the degree of translucency of the endosperm (hence the name "waxy"), and it affects the cooking and eating qualities of the grains and the industrial properties of the starch extracted from those grains.

Because of extensive genetic evidence, it is widely accepted that granule-bound starch synthase (GBSS) activity is a function of the protein coded by the *waxy* gene. The final product of the *waxy* locus is a protein of molecular weight 58,000 associated with the starch granule. This protein can be extracted by heating the starch with SDS or by incubating at 37°C with 9 M urea, but these methods are too drastic for the extraction of starch synthase activity. In mutants containing the *wx* alelle, there is virtually no amylose, GBSS activity is very low (Nelson and Rines, 1962; Tsai, 1974; Nelson *et al.*, 1978), and the waxy protein is missing. One of the two GBSS, partly purified from maize kernels (Macdonald and Preiss, 1983, 1985), had a molecular weight as determined by sucrose density gradients of 60,000.

Shure *et al.* (1983) prepared cDNA clones homologous to *Wx* mRNA. In subsequent experiments (Federoff *et al.*, 1983), restriction endonuclease fragments containing part of the *Wx* locus were cloned from strains carrying the ac *wx*-M9, *wx*-M9, and *wx*-M6 alleles to characterize further the controlling insertion elements activator (ac) and dissociation (ds). Excision of the ds element from the certain wx alleles produces two new alleles (S5 and S9) that are encoding the wx proteins having altered starch synthase activities (Wessler *et al.*, 1986). Two of these, S9 and S5, had 53 and 32% of the starch synthase activity, respectively, seen in the normal endosperm. Mutant

S9, with higher starch synthase activity, had 36% of the amylose content observed in the nonmutant endosperm, whereas mutant S5, with an even lower starch synthase activity of 32%, had only 21% of the nonmutant maize amylose content. These data further support the view that the waxy protein is involved in amylose synthesis.

The DNA sequence of the *Waxy* locus of *Zea mays* was determined by analysis of both a genomic and an almost full-length cDNA clone (Klösgen *et al.*, 1986), and the *Waxy* locus from barley has been cloned and its DNA has been sequenced (Rohde *et al.*, 1988). Figure 1 shows the deduced amino-acid sequences from the maize and barley clones and compares them with the amino-acid sequence for the *E. coli* ADPglucose-specific glycogen synthase (Kumar *et al.*, 1986). Of interest is that 13 of the first 27 amino acids in the *E. coli* glycogen synthase are identical to the amino-acid sequences found in the plant enzymes. Moreover, for the three plant starch synthases, 18 of the first 30 amino acids are identical and others may be considered homologous. The sequence starting at residue Lys 15 of the bacterial enzyme, . . . KTGGL . . ., is particularly significant. The lysine in the bacterial glycogen synthase has been implicated in the binding of the substrate, ADPglucose (Furukawa *et al.*, 1990), due to the chemical modification of that site by the substrate analogue, ADP-pyridoxal. The finding of similarity of sequences between the bacterial glycogen synthase and the putative plant starch synthases provides more and stronger suggestive evidence that the waxy gene is indeed the structural gene for the granule-bound starch synthase.

The complete deduced amino-acid sequences of the open reading frames of the *Waxy* genes from maize and barley are known. About 75% identity can be seen in the sequence with respect to amino acids. If functionally similar amino acids are considered, then the homology is about 81%. Thus, these two proteins are similar in sequence and probably carry out the same function in the starch granule. There is, however, very little sequence homology with the bacterial glycogen synthase beyond the *N*-terminal sequence.

Genetically based evidence indicates that the protein product of the *Wx* gene, the waxy protein, is likely to be a GBSS, and biochemical evidence of the identity of the GBSS and the waxy protein has also been obtained (Sivak *et al.*, 1993). Starch-bound proteins from developing maize endosperm were solubilized by digesting the starch with amylases, fractionated by chromatography, and analyzed by electrophoresis and immunoblot (Sivak *et al.*, 1993). In maize endosperm, there are at least four starch synthases: two soluble (Ozbun *et al.*, 1971) and two granule-bound (Macdonald and Preiss, 1985). The number of isoforms may vary with the plant species and the developmental stage, but those that have been studied more carefully

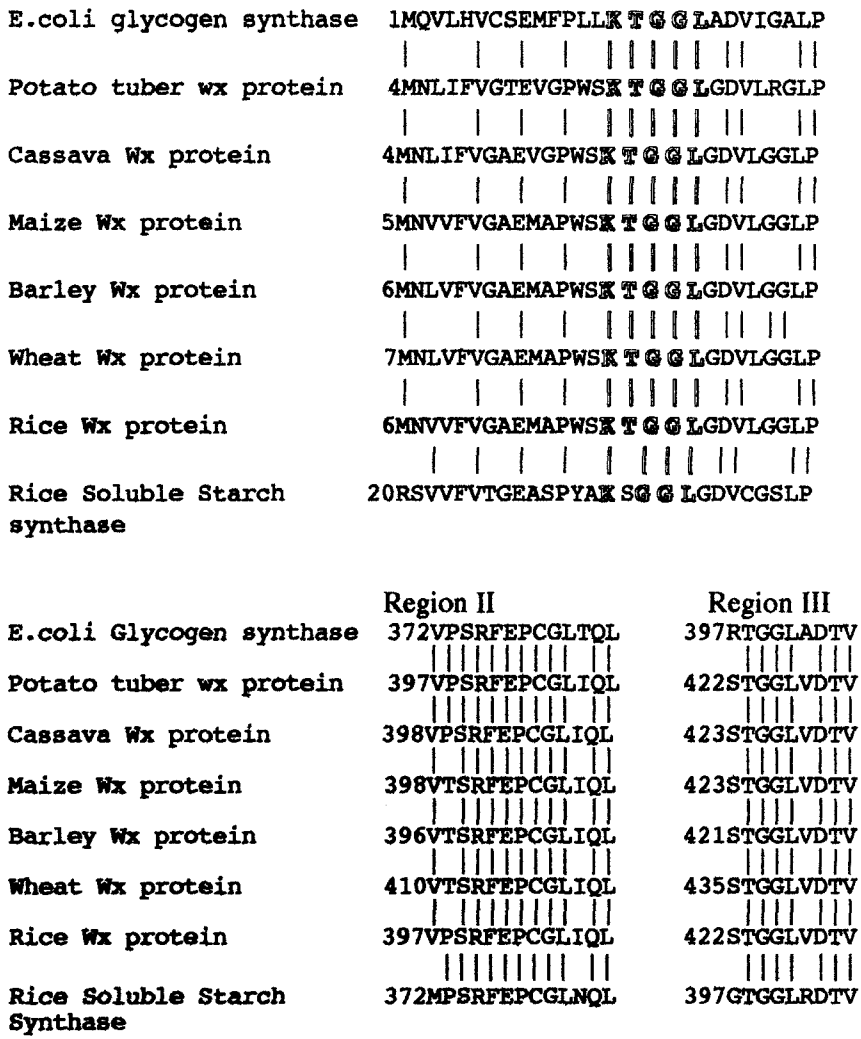

FIG. 1. Regions of amino acids in the sequence of the *E. coli* glycogen synthase that are conserved in granule-bound starch synthases (also known as *Waxy* proteins) and rice seed-soluble starch synthase. The numbers preceding the sequence indicate the residue number from the putative *N*-terminus in the sequence. The sequence in outline form, KTGGL, has been shown for the *E. coli* glycogen synthase to be involved in binding of the sugar nucleotide substrate.

seem to have a similar number of isoforms. Purification of the starch synthase and branching enzymes in large amounts and to a high specific activity has proved to be difficult, and partly for this reason it has not been possible so far to determine how the enzymes interact to produce the two polysaccharides, amylose and amylopectin, that form the starch granule. Starch extracted from developing pea embryos contained starch synthase activity that was associated with the waxy protein, and the molecular weight of the pea starch synthase is about 59,000, as determined by ultracentrifugation in sucrose density gradients. A pea GBSS preparation displayed a relatively high specific activity (more than 10 μmol glucose incorporated per minute per mg protein). When this enzymatic fraction was subjected to SDS polyacrylamide gel electrophoresis and was followed either by protein staining or immunoblot, only the *Wx* protein was visible (Sivak *et al.*, 1993).

Thus, the biochemical examination of starch synthase present in starch granules from two species, maize and pea, strengthens the genetic evidence supporting the role of the *Wx* protein as a GBSS with a major role in the determination of the amylose content of starch. It is still not clear, however, how the loss of starch synthase present in the starch granule causes the disappearane of amylose. It is possible that the interior of the granule is devoid of branching enzyme or, if branching enzyme is in the granule itself, it is not appreciably active. The presence of an active, chain-elongating enzyme (i.e., starch synthase), without an active branching enzyme present (in the presence or absence of some debranching activity), could lead to amylose formation. For a discussion of how the primer is formed, see the chapter, "Open Questions and Hypotheses in Starch."

The starch synthase isozymes in maize endosperm have different molecular masses. The GBSS isozyme I has a molecular mass of 60,000; GBSSII is 95,000. The SSSI has a molecular mass of 72,000, and SSSII is 95,000. Mu *et al.* (1994) have reported the molecular mass of maize endosperm SSSI as 76,000, which is similar to the value reported previously for SSSI (reviewed in Sivak and Preiss, 1994; Preiss and Sivak, 1996). These molecular mass values for the starch synthases are all higher than that of the *E. coli* glycogen synthase with a molecular weight of 52,000 (Kumar *et al.*, 1986).

There is a report that in pea embryo, some of the SSSs may also be bound to the starch granule (Denyer *et al.*, 1993) and that in maize endosperm, some of SSSI adheres to the starch granule (Mu *et al.*, 1994). The conclusions in the pea embryo study (Denyer *et al.*, 1993) are based on positive immunoblots obtained after electrophoresis of the SSS with antibody prepared against the GBSS, and also on the similarity of the amino-acid sequence of three peptides obtained from protease SV8 digests of the SSS. This clearly shows there is a close relationship between SSSII and GBSSII, but does not indicate that they are identical proteins. It is also

not clear how much of the SSSII activity is present as granule-bound activity and how much is soluble activity. It is also not surprising that some of the SSSII is present as starch–granule-bound, as SSSII does have an affinity for its substrate, starch.

The evidence for the maize study rests on the observation of a positive immunoblot with antibody prepared against a 76 kDa protein obtained from the starch granule with the SSSI on electrophoresis; the antibody also neutralized the SSSI activity. Since SSSI has affinity for the granule, one would expect to have some of the SSSI protein bound to the granule, and the question is how much is bound and whether the binding is similar to the binding of the GBSS to the starch granule. There is no question that in maize, the GBSSI is immunologically distinct from the SSSI (reviewed in Sivak and Preiss, 1995; Preiss and Sivak, 1996).

The amino-acid sequences of the bacterial glycogen synthase and the plant starch synthases have been compared (Fig. 1). There are three regions of high conservation and at least one of them is involved in binding the substrate, ADPglucose (Furukawa *et al.*, 1990, 1993). This region (region I) is at the *N*-terminal. The possible functions for regions II and III are not known. However, the high degree of conservation of amino acids in region II (only one or two amino acids, at most, differ from those in the sequence of glycogen synthase of *E. coli*) suggesting that this is likely to be an important site. In region III, all the GBSSs are identical with respect to the amino-acid sequence, whereas the *E. coli* sequence differs in only two of the nine amino acids: Arg for Ser and Ala for Val. The SSS has Gly for that Ser and an Arg residue instead of Val. In addition, Lys residue 277 of the *E. coli* glycogen synthase is also involved in catalysis (Furukawa *et al.*, 1994) and is also conserved in the GBSS and SSS. Many questions remain with respect to protein–structure–function relationships among the three types of α-1,4 glucan synthases and the primer binding site and amino-acid residues involved in catalysis. In rice seed, there is no question that the SSSs are different than the granule-bound starch synthases in that there are only 29 to 37% identities with the rice GBSSs (Baba *et al.*, 1993).

Thus far, the only α-1,4 glucan synthase reported to be overexpressed with high activity is the *E. coli* enzyme. This system should be further exploited with respect to the methodologies of chemical modification, site-directed mutagenesis, and attempts to determine its three-dimensional structure.

V. STUDIES OF *Chlamydomonas reinhardtii* MUTANTS

It is to be expected that different enzymes must have different functions in the synthesis of the starch components, amylose and amylopectin,

and this is what the work with *Chlamydomonas reinhardtii* of Ball and his collaborators (1991) indicates (Delrue *et al.*, 1992; Fontaine *et al.*, 1993; Maddelein *et al.*, 1994). These authors showed that SSSII may be involved in the synthesis of the intermediate sized chains of amylopectin (Fontaine *et al.*, 1993) and that GBSS is not only involved in amylose synthesis, but also in amylopectin synthesis (Maddelein *et al.*, 1994). The process to follow is to separate the various granule-bound and soluble starch synthases from each other and to examine their properties. The properties that must be studied are their specificities (their chain-lengthening properties), to what chains they prefer to transfer glucosyl residues (the A-, B1-, B2-, B3-, or B4-chains of amylopectin; Hizukuri, 1986), as well as the optimal length of glucosyl residues they can synthesize efficiently. Amylose synthesis depends on the concentration of ADPGlc, as GBSS has a high K_m for the substrate as compared to the soluble starch synthases (Van den Koornhuyse *et al.*, 1996). The phosphoglucomutase (PGM)-deficient mutants can make amylopectin but not amylose, as shown by detailed structure studies of the starch accumulated by the algae (Libessart *et al.*, 1995), even though the algae have GBSS. A similar structure effect can be seen when the algae have defective ADPGlc PPase (Van den Koornhuyse *et al.*, 1996).

Ball and associates (1991) have isolated various mutants of *Chlamydomonas* that are deficient in starch synthase activities. These are a GBSS-deficient mutant (Delrue *et al.*, 1992), an SSSII-deficient mutant (Fontaine *et al.*, 1993), and a double mutant—that is, a mutant deficient both in GBSS and in SSSII (Maddelein *et al.*, 1994). The SSSII mutant had only 20 to 40% of the wild-type starch content, and the amylose fraction of the starch increased from 25 to 55%. This mutant also had a modified amylopectin with an increased amount of short chains of DP 2 to 7, and a decrease of intermediate size chains of DP 8 to 60. This suggests that the SSSII is involved in the synthesis or maintenance of the intermediate size chains (mainly B-chains) in amylopectin. The higher amylose content could be explained because of the failure of the SSSII mutant to make extended chains.

The double mutants, defective in SSSII and GBSS (Maddelein *et al.*, 1994), had a starch content of only 2 to 16% of the wild-type. The severity of the GBSS defect of the double mutant dictated the amount of starch present in the double mutant, with an almost null mutant having little starch. The authors suggest that GBSS is important for synthesis of the internal structure of the amylopectin, and the effect of GBSS deficiency is worsened by the diminished SSSII activity.

These studies, using *Chlamydomonas* mutants, provide evidence for the involvement of the GBSS, not only in amylose, but also in amylopectin synthesis, and suggest that a function for SSSII is in the synthesis of the intermediate size B-branch chains in amylopectin.

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

Neuffer, M. G., Coe, E. H., and Wessler, S. R. (1997)