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Formation of callose from sucrose in cotton fiber microsomal membranes

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Abstract Callose is formed from exogenous sucrose by cotton fiber microsomal membranes that contain both sucrose synthase and callose synthase activity. Although the coupled reaction between sucrose and callose synthases occurs predominantly to channel glucose from sucrose-derived uridine diphosphate (UDP)-glucose into callose in the membranes, there is no difference in the UDP-glucose-forming/sucrose-forming activity ratios between the soluble and membrane-bound forms of sucrose synthase. The consumption of UDP-glucose from sucrose into callose probably leads to UDP-glucose formation rather than sucrose formation despite the lower affinity of sucrose synthase for sucrose than for UDP-glucose. Callose formation is markedly stimulated by the addition of either recombinant Glu^{11} (S11E) or in vitro phosphorylated Ser^{11} mung bean sucrose synthase but not by the wild-type nonphosphorylated Ser^{11} enzyme. We propose that a negative charge (by phosphorylation or mutagenesis) at Ser^{11} in sucrose synthase causes the enzyme to promote a coupled callose-forming reaction.

Key words Callose · UDP-glucose · Sucrose synthase · Cotton · Fiber · Sucrose

Introduction

Higher plants have two enzyme systems for the synthesis of uridine diphosphate (UDP)-glucose: one involves UDP-glucose pyrophosphorylase (EC 2.7.7.9) and the other sucrose synthase (EC 2.4.1.13). The latter enzyme catalyzes a freely reversible reaction: sucrose + UDP = UDP-glucose + fructose. The high free energy during formation of sucrose from glucose and fructose can be conserved and used for synthesis of UDP-glucose during sucrose cleavage, whereas two adenosine triphosphate (ATP) equivalents are required for synthesis of UDP-glucose from sucrose in the absence of sucrose synthase. The amount of sucrose synthase is much higher in nonphotosynthetic sink tissues, where sucrose is the main source of carbon that is translocated and cleaved by the enzyme to produce UDP-glucose as a precursor of various polysaccharides. In developing cotton fibers, sucrose synthase is localized in arrays parallel with the helical pattern of cellulose deposition.[1] Hirai et al. reported the formation of β-glucan microfibrils from sucrose and UDP during incubation of tobacco plasma membrane sheets,[2] indicating the presence of sucrose synthase associated with this membrane, where a coupled reaction occurs between sucrose synthase and β-glucan synthase.

Sucrose synthase is possibly regulated posttranslationally by reversible seryl phosphorylation, which may alter its activity by increasing the apparent affinity for UDP or sucrose[3] or its localization by decreasing its association with plant membranes.[4] Comparison of deduced amino acid sequences reveals that sucrose synthases from numerous plant species have a conserved Ser residue for phosphorylation in a related phosphorylation motif, basic-x-x-Ser-hydrophobic near the N-terminus.[5] Phosphorylation occurs at Ser^{15} in the maize SS-2 isoform,[5,6] primarily at Ser^{11} in soybean root nodule sucrose synthase,[7,8] and at Ser^{11} in mung bean sucrose synthase.[3] The apparent K_m value for sucrose of the recombinant mung bean enzyme was decreased by a factor of 2.6 (K_m 161 mM for the wild-type enzyme and K_m 61 mM for the phosphorylated wild-type enzyme) following its in vitro phosphorylation by a nodule Ca^{2+}-dependent protein kinase preparation from soybean nodules,[7] whereas the phosphorylated enzyme's K_m value for UDP-glucose (0.36 mM) was much lower than that for sucrose.[3] It should be noted that the apparent K_m values for UDP-glucose and fructose were similar for the wild-type

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and phosphorylated enzyme forms.[3] Notably, the k_{cat} value of the phosphorylated enzyme with sucrose was about 2.2 times lower than that of the wild-type enzyme, and thus the k_{cat}/K_m ratio of the phosphorylated enzyme was only about 20% higher than that of the wild type. Therefore phosphorylation of the target enzyme did not cause marked activation of its UDP-glucose-forming activity, although the apparent affinity for sucrose was increased. The question of whether this increased affinity of the enzyme for sucrose affects sucrose metabolism in plants clearly warrants resolution.

A mutational analysis of mung bean sucrose synthase was performed by site-directed mutagenesis of the recombinant protein expressed in *Escherichia coli*, in which a Glu residue was introduced at position 11 (S11E mutant).[3] This replacement of Ser^{11} significantly decreased the enzyme's K_m value for sucrose to produce UDP-glucose by a factor of 7.0 (K_m 23 mM). The k_{cat}/K_m ratio, which reflects the overall catalytic efficiency of the S11E mutant enzyme with respect to sucrose, was 3.1 times higher than that of the wild-type enzyme and 2.6 times that of the phosphorylated Ser^{11} enzyme. These findings indicate that the S11E mutant enzyme is functionally superior to phosphorylated sucrose synthase, although the mutant enzyme mimics the phospho enzyme owing to the introduction of an anionic side chain at position 11.

The S11E mutant sucrose synthase enhanced the production of cellulose in *Acetobacter xylinum* when the cells were grown in the presence of sucrose as the carbon source.[9] This enhanced cellulose production was probably due to the low level of UDP in the cells in that the UDP formed after glucosyl transfer could be rapidly and directly recycled back into UDP-glucose by sucrose synthase. Because UDP inhibits cellulose synthase in *A. xylinum* (K_i 0.14 mM)[10] sucrose synthase apparently functions to prevent the buildup of free UDP during cellulose synthesis. In developing cotton fibers, callose and cellulose synthases are likely associated with a plasma membrane-bound form of sucrose synthase.[1] An important but as yet unanswered question is whether the negative charge at position 11 in the S11E mutant enzyme affects its interaction with membrane-bound β-glucan synthase in cotton fibers. This paper describes the characteristics of sucrose synthase activity and the effect of this mutation and the phosphorylation of sucrose synthase (Ser^{11}) on callose synthesis in cotton fiber microsomal membranes.

Materials and methods

Materials

[^{14}C]Sucrose (620 μCi/μmol), UDP-[^{14}C]glucose (290 μCi/μmol), and Aquasol were obtained from New England Nuclear. Seeds of cotton (*Gossypium hirsutum*) were obtained from Takii, and plants were grown in a growth chamber and in the field. Exo-1,3-β-glucanase (*Trichoderma* sp.) and endo-1,4-β-glucanase (*Trichoderma* sp.) preparations, purchased from Megazyme, showed the expected specificity to hydrolyze laminarin and cotton cellulose, respectively. However, prolonged incubation caused exo-1,3-β-glucanase to degrade cellulose and endo-1,4-β-glucanase to degrade laminarin. Dowex AG 1 × 8 and Dowex 50 × 8 were obtained from Bio-Rad.

Preparation of cotton fiber microsomal membranes

Cotton membranes for sucrose and callose synthase preparations were prepared from ovules of cotton fiber cells 20 days after anthesis. Fresh fiber cells were ground in a mortar with 100 mM HEPES/KOH (pH 7.5) buffer containing 2 mM EDTA, 5 mM EGTA, and 1 mM DTT in the presence of washed sand. The homogenate was centrifuged twice at 1000 *g* for 10 min and filtered through nylon mesh. The supernatant was recentrifuged at 100000 *g* for 30 min. The membrane pellet (microsomal fraction) was suspended in 20 mM HEPES/KOH (pH 7.5) and used for the enzyme assay.

Callose synthesis assay

The assay of callose synthase was performed essentially according to the method of Hayashi et al. with some modifications.[11] The reaction mixture contained 50 mM HEPES/KOH (pH 7.5), 5 mM $CaCl_2$, 5 mM $MgCl_2$, 1 mM EDTA, 2 mM laminaribiose, and cotton membrane preparations in the presence of either 0.4 mM UDP-[^{14}C]glucose (290 μCi/μmol) or 20 mM [^{14}C]sucrose (500 μCi/μmol) in a total volume of 100 μl. The mixture was kept at 25°C for 10 min. The reaction was terminated by boiling and subsequent addition of 200 μl of 100% (v/v) ethanol containing 0.5 mM EGTA to give a final ethanol concentration of 66%. Carrier cellulose was added to the mixture, and the entire solution was mixed well and centrifuged. The supernatant fraction was carefully removed. After washing five times with 70% (v/v) ethanol containing 5 mM EGTA and once with a chloroform/methanol (1:1) solution, the radioactivity in the precipitate was counted in Aquasol.

Recombinant sucrose synthases

Recombinant wild-type (Ser^{11}) and mutant (S11E) mung bean sucrose synthases were prepared as reported previously.[3] The in vitro phosphorylation of the recombinant Ser^{11} sucrose synthases was performed with a soluble approximately 55-kDa Ca^{2+}-dependent protein kinase (CDPK) preparation partially purified from soybean root nodules.[7] In 40 μl of reaction mixture, about 10 μg of purified wild-type sucrose synthase was incubated with the appropriate amount of nodule CDPK, 50 mM HEPES-KOH pH 7.5, 5 mM $MgCl_2$, and 2 mM ATP for 30 min at 30°C in the presence of 1 mM Ca^{2+}.

Sucrose synthase activity assay

The UDP-glucose-forming activity was measured at pH 7.5 with 100 mM [^{14}C]sucrose (2.5 Ci/mol), 10 mM UDP, and

enzyme protein (10μg) in a total volume of 20μl. Sucrose-forming activity was measured at pH 7.5 with 1mM UDP-[^{14}C]glucose (290Ci/mol), 50mM fructose, and enzyme protein (10μg) in a total volume of 20μl. The reactions were allowed to proceed for 30min at 30°C to form UDP-glucose and for 5min at 30°C to form sucrose. The reactions were terminated by boiling and then spotted on a 2cm wide Whatman 3MM filter paper strip followed by electrophoresis in 50mM sodium tetraborate (pH 9.6) at 250V for 3h.[3] After electrophoresis the paper strips were dried and analyzed by autoradiography or bioimaging on a Fujix Bas 2000 (Fuji Photo Film). The areas corresponding to sucrose, UDP-glucose, and fructose on the paper strip were excised and neutralized, and their ^{14}C-radioactivity was determined by liquid scintillation spectroscopy.

The reaction mixture for nonradioactive assay of UDP-glucose-forming activity contained 100mM sucrose, 10mM UDP, enzyme preparations, and 18mM Tris/HCl (pH 7.5) in a total volume of 50μl. The mixture was incubated for 30min at 30°C, and the amount of fructose was determined by the Nelson-Somogyi method.[12] The reaction mixture for the assay of sucrose-forming activity contained 10mM UDP-glucose, 50mM fructose, enzyme preparation, and 18mM Tris/HCl (pH 7.5) in a total volume of 50μl. After incubation at 30°C for 30min, invertase (0.02 unit) in 20μl 150mM sodium acetate buffer (pH 5.0) was added, and the mixture was incubated at 37°C for 5min. The amount of glucose was determined by the glucose oxidase method after adding 1ml glucose oxidase solution (Glucose C-II test kit; Wako, Japan). One unit of sucrose synthase activity is defined as the amount of enzyme that generates 1μmol fructose or glucose per minute under standard conditions. The K_m value of sucrose synthase for sucrose was estimated from the Lineweaver-Burk plots of reactions. The reaction mixtures contained a total volume of 20μl.

Product analysis

For enzymatic degradation, β-glucan was digested with exo-1,3-β-glucanase (24 units) in 17mM sodium acetate buffer (pH 4.0) or with endo-1,4-β-glucanase (75 units) in 17mM sodium acetate buffer (pH 4.5) for 24h at 37°C. A few drops of chloroform were added to the reaction mixture to prevent bacterial growth.

For chemical (Smith) degradation, β-glucan was incubated at 4°C for 96h in the dark with 30mmol of sodium metaperiodate in 100mM sodium acetate (pH 5.6) in a total volume of 1ml. The reaction mixture was then reduced chemically by adding 100mmol sodium borohydride. After a 2-h incubation, the reaction was stopped by adding trifluoroacetic acid to a final concentration of 2M, and the sample was hydrolyzed by heating at 100°C for 2h. The mixture was taken to dryness to remove trifluoroacetic acid and deionized with Dowex 50W (H^+). Borate was removed by the addition of methanol and evaporation, and the sample was subjected to paper chromatography with 1-propanol/ethyl acetate/water (3:2:1, v/v).

General methods

DNA and amino acid sequence homology searches were performed at the National Center for Biotechnology Information with the BLAST network service. Carbohydrate was determined by the phenol/sulfuric acid method.[13] Protein was quantitated by the modified method of Lowry[14] with DC protein assay (Bio-Rad) and bovine serum albumin as the standard.

Results and discussion

Enzyme distribution in cotton fiber cells

As shown in Table 1, β-glucan was formed not only from UDP-glucose but also from sucrose in a crude, 100000g microsomal fraction isolated from cotton fiber extracts. Detectable levels of both the UDP-glucose- and sucrose-forming activities of sucrose synthase were also present in this fraction, although higher levels were present in the 100000g supernatant (soluble fraction). In addition, both sucrose synthase and β-glucan synthase showed high activity in the 100000g pellet (microsome fraction). Because β-glucan synthase is a marker enzyme for plasma membranes,[15] the substantial activity of sucrose synthase in

Table 1. Distribution of sucrose and β-glucan synthases in the homogenates of cotton fibers

Fraction	Volume (ml)	Protein (mg)	Sucrose synthase activity (nmol/min)		β-Glucan synthase activity (nmol/min)	
			UDP-glucose-forming	Sucrose-forming	From sucrose	From UDP-glucose
Homogenate	15	118	125	890	13.70	2.20
Cell walls	23	202	61	430	23.10	7.10
Supernatant (100000g)	14	73	75	536	0.12	0.05
Pellet (100000g)	0.8	12	11	78	7.60	2.80

Fresh fibers from five ovules were ground in a mortar with 100mM HEPES/KOH (pH 7.5) buffer containing 2mM EDTA, 5mM EGTA, and 1mM DTT in the presence of sand. The homogenate was centrifuged at 1000g for 10min to obtain supernatant and cell wall fractions. The supernatant fraction was centrifuged at 100000g for 30min, and the resulting pellet was suspended in 50mM HEPES/KOH (pH 7.5) buffer. The cell wall fraction was also suspended in the buffer

this wall fraction is likely due to a membrane-bound sucrose synthase associated with plasma membranes inseparable from cell walls during fractionation of cotton fibers. Table 1 shows that about 55% of the total sucrose synthase activity is associated with the combined microsomal and cell wall fractions, which are likely involved in β-glucan synthesis in developing cotton fibers.[1] In addition, the UDP-glucose-forming/sucrose-forming activities ratio was similar in the homogenate, cell wall, high-speed supernatant, and microsomal fractions. The UDP-glucose-forming/sucrose-forming activities ratio was similar in all fractions, whereas phosphorylation of sucrose synthase increased the UDP-glucose-forming/sucrose-forming activity ratio 1.2-fold[3,4] indicating that the level of phosphorylation is similar between membrane and soluble fractions. Thus, the soluble and membrane-bound enzymes may contain the two forms, which are both dephosphorylated and phosphorylated.

β-Glucan synthase activity might directly use sucrose rather than UDP-glucose, although the K_m value for UDP-glucose (0.2 mM) was lower than that for sucrose (12 mM). Nevertheless, β-glucan synthesis was inhibited by UDP (K_i 65 μM) which was derived from UDP-glucose. Although the cell wall fraction contained relatively high levels of contaminating sucrose synthase and β-glucan synthase activities, microsomal membrane preparations were employed for the synthesis of β-glucan to characterize the UDP/UDP-glucose-coupled reaction between sucrose and β-glucan synthases.

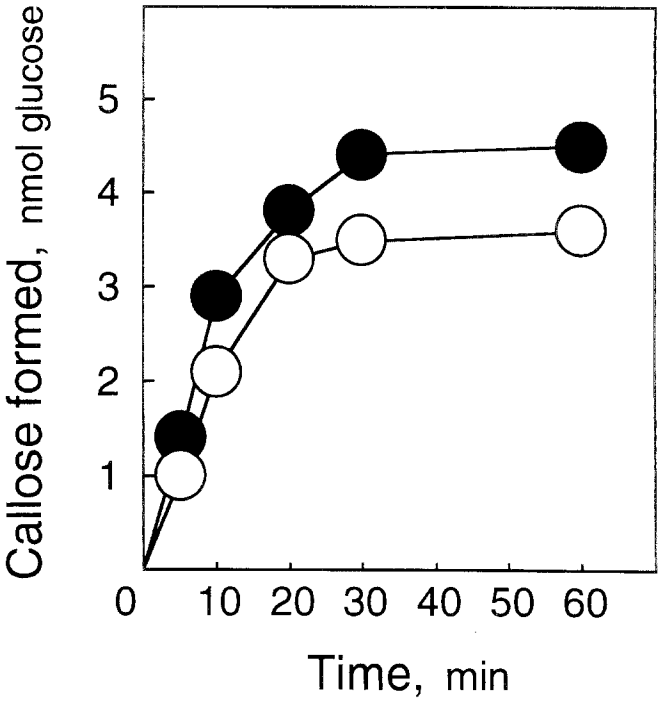

Fig. 1. Time course of callose formation from sucrose by a cotton fiber microsomal preparation. *Closed circles*, callose formed from 20 mM sucrose; *open circles*, callose formed from 20 mM sucrose plus 0.1 mM UDP. The same amount of membrane preparation was used for each reaction

β-Glucan synthesis from sucrose

The incorporation of sucrose-derived glucose into β-glucan by the microsomal membranes gradually increased for 30 min after addition of sucrose (Fig. 1). UDP-glucose was transiently formed from sucrose by sucrose synthase because the K_m value of callose synthase for UDP-glucose (K_m 0.2 mM) is somewhat lower than that of the sucrose-forming activity of sucrose synthase for UDP-glucose (K_m 0.36 mM).[3,11] The consumption of UDP-glucose from sucrose into callose probably leads to sustained UDP-glucose formation by sucrose synthase rather than reverse sucrose formation despite the much lower apparent affinity of sucrose synthase for sucrose than for UDP-glucose. This is the most important aspect of the coupled reaction between sucrose and callose synthases. The overall K_m value for sucrose during β-glucan synthesis was determined to be 12 mM, which is similar to that of plant sucrose synthase for sucrose (15 mM).[16] Addition of UDP (0.1 mM) neither stimulated β-glucan synthesis (Fig. 1) nor increased the apparent affinity for sucrose, probably because the reaction mixture contained endogenous UDP at 0.5 μM concentration. This suggests that sufficient endogenous UDP is present in the membranes, where the nucleotide can be recycled in the UDP/UDP-glucose coupled reaction between sucrose and β-glucan synthases.[1,9] However, inhibition of β-glucan synthesis was observed after addition of excess UDP (K_i 65 mM).

The β-glucan formed from sucrose was largely resistant to endo-1,4-β-glucanase digestion, during which only 13% of the total radioactivity was solubilized. Digestion with exo-1,3-β-glucanase solubilized 81% of the radioactivity, which was mainly composed of glucose. These results are in agreement with those for the enzymatic digestion of β-glucan synthesized from UDP-[^{14}C]glucose.[11] Smith degradation analysis revealed that the sucrose-derived β-glucan produced glucose as the major product (Fig. 2), indicating the predominance of 1 → 3 linkages. Some erythritol was also present, suggesting the presence of 1 → 6 and 1 → 4 linkages. Fluorescence microscopy also revealed that the sucrose-derived β-glucan stained with aniline blue, confirming that it was mostly composed of callose (1,3-β-glucan) (data not shown).

Effect of recombinant sucrose synthases

Callose synthesis from exogenous sucrose in cotton microsomal membranes was markedly increased by adding 0.5 μg of either mutant S11E or the in vitro phosphorylated form of Ser[11] sucrose synthase expressed in *Escherichia coli* but not by adding the wild-type (Ser[11]) enzyme (Table 2). The in vitro phosphorylated enzyme increased incorporation of glucose into callose 3.5-fold, although the phosphorylation event increased its catalytic efficiency (k_{cat}/K_m) for UDP-glucose formation by only 20%.[3] Callose formation was enhanced six-fold by the mutant Glu[11] enzyme, whereas its catalytic efficiency with sucrose was only threefold higher than that of the recombinant wild-type enzyme. The result shows that callose synthesis from exogenous sucrose in cotton microsomal membranes is activated by phospho-

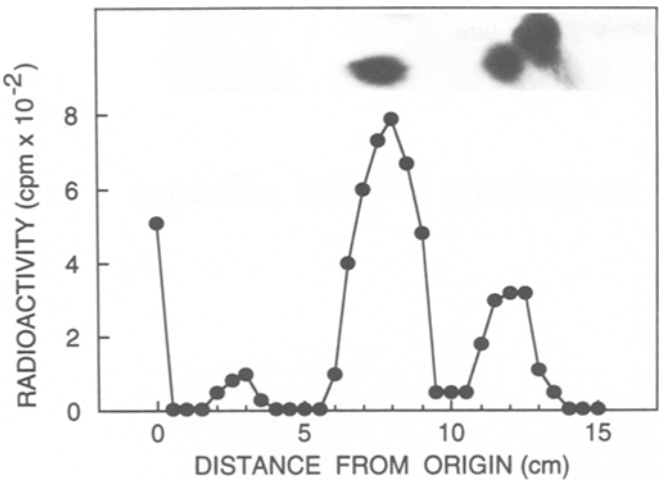

Fig. 2. Paper chromatography of the products generated by Smith degradation of [^{14}C]sucrose-derived β-glucan. The paper was excised every 5 mm, and its radioactivity was counted with Aquasol. The radioactivity is shown by *circles*; and the chromatogram of glucose, erythreitol, and glycerol is shown at the top of the figure. The sugars were stained with alkaline silver nitrate

Table 2. Effect of recombinant sucrose synthases on sucrose-derived callose formation in cotton microsomal membranes

Sucrose synthase (0.5μg)	Callose formed (pmol)
None	2
Ser^{11} (wild-type)	2
In vitro phosphorylated Ser^{11}	7
S11E (mutant)	12

SE is ± 10%

rylated Ser^{11} and mutant Glu^{11} sucrose synthases, which serve to channel carbon directly from sucrose to callose by UDP-glucose/UDP coupling. These exogenous sucrose synthases might act in concert with microsomal callose synthase rather than the exogenous wild-type cotton enzyme (Table 1). The negative charge [Glu (1^-) or phosphorylated Ser (2^-)] at position 11 probably promotes the sucrose synthase/β-glucan synthase coupled reaction. Therefore, phosphorylation of sucrose synthase near its N-terminus may enhance the formation of UDP-glucose in concert with membrane-bound callose synthase in plant tissues.

Because the levels of sucrose synthase mRNA and protein are relatively constant in cotton fibers during all stages of growth,[17] phosphorylation may increase the UDP-glucose-forming activity of sucrose synthase, accompanied with an increase in callose synthase activity at the onset of secondary wall synthesis.[18] The phosphorylation of sucrose synthase may also be involved in cellulose biosynthesis in cotton fibers at the time of secondary wall synthesis, although it is difficult to demonstrate experimentally the reaction coupled with the activity of cellulose synthase.

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References

1. Amor Y, Haigler CH, Johnson S, Wainscott M, Delmer DP (1995) A membrane-associated form of sucrose synthase and its potential role in synthesis of cellulose and callose in plants. Proc Natl Acad Sci USA 92:9353–9357
2. Hirai N, Sonobe S, Hayashi T (1998) In situ synthesis of β-glucan microfibrils on tobacco plasma membrane sheets. Proc Natl Acad Sci USA 96:14–18
3. Nakai T, Konishi T, Zhang X-Q, Chollet R, Tonouchi N, Tsuchida T, Yoshinaga F, Mori H, Sakai F, Hayashi T (1998) An increase in apparent affinity for sucrose synthase of mung bean sucrose synthase is caused by in vitro phosphorylation or directed mutagenesis of Ser^{11}. Plant Cell Physiol 39:1337–1341
4. Winter H, Huber JL, Huber SC (1997) Membrane association of sucrose synthase: change during the graviresponse and possible control by protein phosphorylation. FEBS Lett 420:151–155
5. Huber SC, Huber JL, Liao PC, Gage DA, McMichael RW Jr, Chourey PS, Hannah LC, Koch K (1996) Phosphorylation of serine-15 of maize leaf sucrose synthase. Plant Physiol 112:793–802
6. Shaw JR, Ferl RJ, Baier J, St. Clair D, Carson C, McCarty DR, Hannah LC (1994) Structural features of the maize sus1 gene and protein. Plant Physiol 106:1659–1665
7. Zhang X-Q, Chollet R (1997) Seryl-phosphorylation of soybean noudle sucrose synthase (nodulin-100) by a Ca^{2+}-dependent protein kinase. FEBS Lett 410:126–130
8. Zhang X-Q, Verma DPS, Patil S, Arredondo-Peter R, Miao G-H, Kuismanen R, Klucas RV, Chollet R (1997) Cloning of a full-length sucrose synthase cDNA from soybean (*Glycine max*) root nodules (accession no. AFO30231) (PGR97-173). Plant Physiol 115:1729
9. Nakai T, Tonouchi N, Konishi T, Kojima Y, Tsuchida T, Yoshinaga F, Sakai F, Hayashi T (1999) Enhancement of cellulose production by expression of sucrose synthase in *Acetobacter xylinum*. Proc Natl Acad Sci USA 96:14–18
10. Benziman M, Aloni Y, Delmer DP (1983) Unique regulatory properties of the UDP-glucose: 1,4-β-D-glucan synthase of *Acetobacter xylinum*. J Appl Polym Sci 37:131–143
11. Hayashi T, Read SM, Bussell J, Thelen M, Lin FC, Brown RM Jr, Delmer DP (1987) UDP-glucose: (1 → 3)-β-glucan synthases from mung bean and cotton. Plant Physiol 83:1054–1062
12. Somogyi M (1952) Notes on sugar determination. J Biol Chem 195:19–23
13. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
15. Van Der Woude W, Lembi CA, Morre DJ, Kindinger JI, Ordin L (1974) β-Glucan synthetases of plasma membrane and Golgi apparatus from onion stem. Plant Physiol 54:333–340
16. Delmer DP (1972) The purification and properties of sucrose synthase from etiolated *Phaseolus aureus* seedlings. 247:3822–3828
17. Shimizu Y, Aotuka S, Hasegawa O, Kawada T, Sakuno T, Sakai F, Hayashi T (1997) Change in levels of mRANs for cell wall-related enzymes in growing cotton fiber cells. Plant Cell Physiol 38:375–378
18. Delmer DP, Cooper G, Alexander D, Cooper J, Hayashi T, Nitsche C, Thelen M (1985) New approaches to the study of cellulose biosynthesis. J Cell Sci 2(Suppl):33–50