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An Expression of the Sweet Potato *GBSSI* Gene in *Escherichia coli*

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ABSTRACT: A starch synthase cDNA clone (*spss67*) was isolated from tuberous roots of sweet potato. This clone contained an open reading frame corresponding to a protein of 608 amino acid residues, which also included three consensus regions of starch synthases. Amino acid sequence analyses showed that the protein product of *spss67* shared a high degree of homology with other plant granule-bound starch synthase I (GBSSI). Comparisons of N-terminal amino acid sequence of *spss67* cDNA protein product with that of the native protein isolated from sweet potato indicated that the protein contained a signal peptide of 77 amino acids, and the transit peptide had hydrophobic regions in the initiation and end of peptide that was also present in GBSSI of other plant species. In addition, the protein product of *spss67* expressed in *E. coli* could be recognized by anti-GBSSI antibody of potato. Moreover, this protein exhibited a high activity of starch synthase when amylose was used as a primer, but the activity was not significant when the primer was replaced by amylopectin or glycogen. Studies based on the sequence analyses, western blotting and priming activity indicated that the product of *spss67* clone was a GBSSI.

KEY WORDS: cDNA clone, Starch synthase, Amylose, Amylopectin, Transit peptide, Transformant.

INTRODUCTION

Starch is a polymer complex consisting of amylose and amylopectin. In general, the ratio of amylose to amylopectin is about 1:3, but the ratio may vary in different plant species (Manners, 1985). Starch synthase is involved in the synthesis of amylose and amylopectin, and two different forms are recognized based on their localizations in the amyloplast. Granule-bound starch synthase (GBSS), also known as the WAXY protein, is tightly bound in starch granules and is responsible for amylose synthesis (Tsai, 1974). The activity of another form is found in the soluble fraction of amyloplasts, and the protein is named as soluble starch synthase (SSS) (Preiss, 1988). Biochemical properties of GBSS and SSS are different. GBSS transfers glucose unit from ADP-glucose or UDP-glucose to nonreducing ends of α-1,4 glucose polymers although the rate of transfer from ADP-glucose is higher than that from UDP-glucose; in contrast, SSS uses ADP-glucose as the sole substrate (Tsai, 1974). In addition, GBSS prefers amylose over amylopectin as a primer while SSS would only add the glucose unit into amylopectin (Tsai, 1973). The function of SSS is still not well known, although several studies indicated that mutations on the *SSS* gene seem to affect the arrangement of short and long branches of starch in granules (Martin and Smith, 1995; Nelson and Pan, 1995; Smith *et al.*, 1997). Comparisons of amino acid sequences of various starch synthases revealed three conserved regions, and the first region contained a putative substrate binding site, Lys-X-Gly-Gly (KXGG; X represents a not conserved residue), which was similar to the substrate binding site of glycogen synthase of *Escherichia coli* (*E. coli*) (Furukawa *et al.*, 1994).

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GBSS may be further classified into several types according to their molecular weights and distributions in amyloplasts. GBSSI is found tightly bound to starch granules and has a molecule mass of 58-60 kDa. This type of GBSS provides the largest proportion of total GBSS activity (Shure *et al.*, 1983; Vos-Scheperkeuter *et al.*, 1986; Dry *et al.*, 1992). The *waxy* mutants of maize, potato and barley all lack the activity of GBSSI (Nelson and Tsai, 1964; Tsai, 1974; Hovenkamp-Hermelink *et al.*, 1987; Hylton *et al.*, 1996). The second type, GBSSII (or called SSII), has a molecular mass of 77-79 kDa, and it could be found in starch granules as well as in the soluble fraction (Dry *et al.*, 1992; Denyer *et al.*, 1995; Edwards *et al.*, 1996; Hylton *et al.*, 1996; Craig *et al.*, 1998). The role of GBSSII has not been established; however, it was suggested that GBSSII might play a role in affecting amylopectin structure and starch granule morphology in pea (Craig *et al.*, 1998). The third type of GBSS was named SSIII and it was purified from potato tubers with a molecular mass of 140 kDa (Marshall *et al.*, 1996). The activity of SSIII was found primarily in the soluble fraction of amyloplasts, but it could also bind to starch granules. Studies with transgenic potato deficient in SSIII protein indicated that these potato did not change the ratio of amylose to amylopectin, but the branching pattern of amylopectin was different from that of the wild type (Abel *et al.*, 1996; Marshall *et al.*, 1996; Edwards *et al.*, 1999).

Although GBSSI (WAXY protein) is a major enzyme responsible for amylose synthesis, little is known about regulations of this gene in different tissues. As the first step to study regulations of *GBSSI* gene in the sink and source tissues, we isolated a cDNA clone (*spss67*) from tuberous roots of sweet potato using a partial DNA fragment of starch synthase gene as a probe that contained a sequence encoding the putative substrate binding site (Wang *et al.*, 1999). The protein product of *spss67* cDNA was expressed in *E. coli* and characterized according to sequence alignments and biochemical assays.

MATERIALS AND METHODS

Isolation of starch granules and GBSS enzyme

Tuberous roots (2 g) of sweet potato (*Ipomoea batatas* Lam. cv. Tainong 57) were powdered in liquid nitrogen with a mortar and pestle. Subsequently, the powder was ground with 10 ml of buffer (50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 2 mM DTT) before filtering through 2 layers of Miracloth (Calbiochem, USA) and centrifuging at 12,000*g* for 10 min at 4 ℃. The pellet including starch granules was washed 3 times with ice-cold extraction buffer following by 4 times with cold acetone (Tsai, 1974). For extracting starch granules from the leaf tissue, leaves (5 g) were powdered in liquid nitrogen and subsequently ground with 25 ml of extraction buffer (50 mM Tris-HCl, pH7.4, 2 mM EDTA and 1mM DTT). The sample mixture was filtered through two layers of Miracloth (Calbiochem, USA) before centrifuging at 5,000*g* for 5 min. The pellet was then resuspended with 0.5 ml of gradient buffer (50 mM Tris-HCl, pH7.4, 2 mM EDTA, 1mM DTT and 350 mM sorbitol), and loaded onto a discontinuous Percoll density gradient solution (80% Percoll, 0.6 mM GSH, 2.4% [w/v] PEG 4000, 0.8% [w/v] BSA, 0.8% [w/v] Ficoll, 20% [v/v] 5× gradient buffer). After centrifuging at 7,000*g* for 15 min, the starch granule pellet was washed by cold acetone and then air-dried.

Western blot analysis and sequencing of N-terminus of GBSSI protein

For SDS-polyacrylamide gel analyses, starch granule-bound proteins were extracted by boiling 15 mg of starch granules with 100 μl of SDS-sample buffer (20 mM Tris-HCl, pH 8.0,

2 mM EDTA, 20% glycerol, 2% SDS, 10% β-mercaptoethanol) (Salehuzzaman *et al.*, 1993). Proteins were separated on a 7.5% SDS-polyacrylamide gel and transferred to nitrocellulose membrane (Amersham, UK) for western blot analysis using an antibody raised against the potato GBSSI (kindly provided by Dr R. Visser). For N-terminal sequencing of the GBSSI protein, GBSSI was purified from starch granules by separation on 7.5% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, USA). GBSSI was excised from the membrane and the N-terminal amino acid sequence was analyzed using a Perkin Elmer Applied Biosystem 477A protein sequenator.

Activity assay of GBSSI

GBSSI activity was assayed according to the method described by Vos-Scheperkeuter *et al.* (1986). Two mg starch granules were suspended in 50 μL of a reaction mixture containing 100 mM Bicine, pH 8.5, 5 mM EDTA, 10 mM GSH, 25 mM potassium acetate, 1 mM ADP-glucose and 42.65 kBq ADP-^{14}C glucose (Amersham, 10.5 GBq mmol^{-1}). The mixture was incubated at 37℃ before the reaction was stopped by adding 1 ml of precipitation mix (25% [v/v] H_2O, 75% [v/v] methanol and 1% [w/v] KCl) and then allowed to stand in an ice box for 10 min. Starch was collected by centrifugation, and washed twice by H_2O with each wash precipitated by the precipitation mix. Finally, starch was resuspended in 300 μL of H_2O and the radioactivity was counted using a liquid scintillation counter.

Expression of GBSSI in *E. coli*

The coding region of *GBSSI* cDNA was amplified by PCR using the following primer pairs: sense primer [5'-TCGGATCCATGGCGACTATAACTGC-3'], and antisense primer [5'-TCGAATTCGGTGGAGTAGCGACGTT-3']. The amplified fragment was constructed into *Bam*HI-*Eco*RI cloning sites of pET-21a vector (Novagen, USA). This constructed plasmid was transferred into *E. coli* strain BL21 (DE3) and incubated in Luria broth (LB) medium containing 50 μg ampicillin ml^{-1} as a selective antibiotic. Cells were grown to an absorbency of 0.6 at 600 nm before expression of the target gene was induced by 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 3 hr. For the extraction of starch synthase, 2 ml of culture cells were harvested and lysed by sonication in 200 μL of extraction buffer (50 mM Tris-HCl, pH 7.4 and 2 mM EDTA). The homogenate was centrifuged at 10,000*g* for 10 min. The pellet was washed three times with extraction buffer and used for western analysis and assaying the starch synthase activity. For the assay of starch synthase activity, the pellet was resuspended in the reaction buffer containing primers (1.5 mg of amylose, amylopectin or glycogen) as previously described.

RESULTS AND DISCUSSION

Peptide sequence alignments between sweet potato starch synthase and those of other plant species

A starch synthase cDNA clone, *spss67* (accession number, U44126), was isolated from tuberous roots of sweet potato (Wang *et al.*, 1999). Southern analysis indicated that only one copy of this gene was present in the sweet potato genome (Wang *et al.*, 1999). A 67-kDa protein could be deduced from the ORF of the *spss67* clone, and the deduced amino acid sequence of *spss67* showed 65% to 80% identity to GBSSI of other species; however, the sequence had low homology with the GBSSII, SSIII of potato and SSS of rice. Comparisons of amino acid sequences indicated that the product of *spss67* could be a GBSSI protein (Wang

et al., 1999). Alignments of the deduced amino acid of *spss67* with those of other species showed that the protein product of *spss67* also contained three consensus domains (BOX 1, 2 and 3) of starch synthase (Fig. 1). The first region contained the putative substrate-binding

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                                                                                MATAAGM GIGAACLVAP QVRPGRRLRL QRVRRRCVAE LSRDGGSAHG
rice/SSS

sweet potato ..........  .....MATIT ASHFV...SH VCGGATSGES KVGLGQLAL. RSQAVTHNGL RPVNKIDMLQ LRTSARNLAK
cassava      ..........  .....MATVI AAHFVSRSSH LSTHALETKA N.NLSHTGP. WTQTITPNGL RSLNTMDKLQ MKTQSKAVKK
pea          ..........  .....MATIT GSSMPTRTAC FNYQGRSAES KLNLPQIHFN NNQAFPVLGL RSLN...KLH VRT...ARAT
barly        ..........  .....MAALA TSQLATSGTV LGVTDR.... .FRRPGF... ..QGLRPRNP A..DAALGMR TIGASAAPKQ
wheat        ..........  .....MAALV TSQLATSGTV LSVTDR.... .FRRPGF... ..QGLRPRNP A..DAALGMR TVGASAAPKQ
rice/GBSS    ..........  .....MSALT TSQLATSATG FGIADRSAPS SLLRHGF... ..QGLKPRSP AGGDATSLSV TTSARATPKQ
rice/SSS     PLAPAPLVKQ PVLPTFLVPT STPPAPTQSP APAPTPPPLP DSGVGEIEPD LEGLTEDS.. .........I DKTIFVASEQ

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sweet potato MEGKMRVEWQ AGTIVCKQ.. .QGMNLVFVG CEEGPWCKTG GLGDVLGGLP PALAARGHRV MTVCPRY... ...DQYKDAW
cassva       VSATGN.GRP AAKIIC.G.. .HGMNLIFVG AEVGPWSKTG GLGDVLGGLP PAMAARGHRV MTVSPRY... ...DQYKDAW
pea          SGSSDTSEKS LGKIVC.... ..GMSLVFVG AEVGPWSKTG GLGDVLGGLP PVLAGNGHRV MTVSPRY... ...DQYKDAW
barly        SRKAHRGSRR CLSVVVSATG S.GMNLVFVG AEMAPWSKTG GLGDVLGGLP PAMAANGHRV MVVSPRY... ...DQYKDAW
wheat        SRKPHRFDRR CLSMVVRATG SGGMNLVFVG AEMAPWSKTG GLGDVLGGLP AAMAANGHRV MVISPRY... ...DQYKDAW
rice/GBSS    QRSVQRGSRR FPSVVVYATG A.GMNVVFVG AEMAPWSKTG GLGDVLGGLP PAMAANGHRV MVISPRY... ...DQYKDAW
rice/SSS     ESEIMDVKEQ AQAKV....T RSV...VFVT GEASPYAKSG GLGDVCGSLP IALALRGHRV MVVMPRYMNG ALNKNFANAF
                                                                     BOX 1
sweet potato ETCVVVEPQV GDRIEPVRFF HSYKRGVDRV FVDHPMFLEK VWGKTGSMLY GPKAGKDYKD NQLRFSLLCQ AALEAPRVLN
cassava      DTSVSVEIKI GDRIETVRFF HSYKRGVDRV FVDHPMFLEK VWGKTGSKIY GPRAGLDYQD NQLRFSLLCL AALEAPRVLN
pea          DTNVLVEVKV GDKIETVRFF HCYKRGVDRV FVDHPLFLER VWGKTGSKLY GPKTGIDYRD NQLRFSLLCQ AALEAPRVLN
barly        DTSVISEIKV ADEYERVRFF HCYKRGVDRV FIDHPWFLEK VRGKTKEKIY GPDAGTDYED NQQRFSLLCQ AALEAPRILN
wheat        DTSVISEIKV VDRYERVRYF HCYKRGVDRV FVDHPCFLEK VRGKTKEKIY GPDAGTDYED NQQRFSLLCQ AALEVPRILD
rice/GBSS    DTSVVAEIKV ADRYERVRFF HCYKRGVDRV FIDHPSFLEK VWGKTGEKIY GPDTGVDYKD NQMRFSLLCQ AALEAPRILN
rice/SSS     YTEKHIKIPC FGGEHEVTFF HEYRDSVDWV FVDHPSY... ..HRPG.NLY GDNFG.AFGD NQFRYTLLCY AACEAPLILE

sweet potato LNSSKYFSGP Y..........  ..GEDVVFVA NDWHTALLPC YLKTMYQSRG IYMNAKVAFC IHNIAYQGRF AFSDFSLLNL
cassava      LNSSKNFSGP Y..........  ..GEEVAFIA NDWHTALLPC YLKAIYQPMG IYKHAKVAFC IHNIAYQGRF AFSDFPRLNL
pea          LNSSKYFSGP Y..........  ..GEDVIFVA NDWHSALIPC YLKSMYKSRG LYKNAKVAFC IHNIAYQGRN AFSDFSLLNL
barly        LNNNPYFSGP Y..........  ..GEDVVFVC NDWHTGLLAC YLKSNYQSNG IYRTAKVAFC IHNISYQGRF SFDDFAQLNL
wheat        LNNNPHFSGP YAMLCRAVPR RAGEDVVFVC NDWHTGLLAC YLKSNYQSNG IYRTAKVAFC IHNISYQGRF SFDDFAQLNL
rice/GBSS    LNNNPYFKGT Y..........  ..GEDVVFVC NDWHTGPLAS YLKNNYQPNG IYRNAKVAFC IHNISYQGRF AFEDYPELNL
rice/SSS     LGGYIY.... ..........  .GQKCM.FVV NDWHASLVPV LLAAKYRPYG VYRDARSVLV IHNLAHQGVE PASTYPDLGL

sweet potato PDEYKGSFDF IDGY....DK FVKGRKINWM KAGIREADRV FTVSPNYAKE LVSCVSKGVE LDNHI..RDC GITGICNGMD
cassava      PDKFKSSFDF IDGY....EK FVKGRKINWM KAGILESDRV LTVSPYYAQE VISGVERGVE LDNFI..RKT GIAGIINGMD
pea          PDEFRSSFDF IDGY....NK PCEGKKINWM KAGILESDQV FTVSPHYAKE LISGEDRGVE LDNII..RST GIIGIVNGMD
barly        PDRFKSSFDF IDGY....DK FVEGRKINWM KAGILQADKV LTVSPYYAEE LISGEARGCE LDNIM..RLT GITGIVNGMD
wheat        PDRFKSSFDF IDGY....DK FVEGRKINWM KAGILQADKV LTVSPYYAEE LISGEARGCE LDNIM..RLT GITGIVNGMD
rice/GBSS    SERFRSSFDF IDGY....DT FVEGRKINWM KAGILEADRV LTVSPYYAEE LISGIARGCE LDNIM..RLT GITGIVNGMD
rice/SSS     PPEWYGALEW VFPEWARRHA LDKGEAVNFL KGAVVTADRI VTVSQGYSWE VTTAEG.GQG LNELLSSRKS VLNGIVNGID

sweet potato TQEWNPATDK YLA.....VK YDITTVMQAK PLLKEALQAA VGLPVDRNIP LIGFIGRLEE QKGSDILYAA ISKFI.SMDV
cassava      VQEWNPVTDK YID.....IH YDATTVMDAK PLLKEALQAE VGLPVDRNVP LIGFIGRLEE QKGSDIFVAA ISQLV.EHNV
pea          NREWSPQTDR YID.....VH YNETTVTEAK PLLKGTLQAE IGLPVDSSIP LIGFIGRLEE QKGSDILVEA IAKFA.DENV
barly        VSEWDPTKDK FLA.....VN YDITTALEAK ALNKEALQAE VGLPVDRKVP LVAFIGRLEE QKGPDVMIAA IPEILKEEDV
wheat        VSEWDPIKDK FLT.....VN YDVTTALEGK ALNKEALQAE VGLPVDRKVP LVAFIGRLEE QKGPDVMIAA IPEIVKEEDV
rice/GBSS    VSEWDPSKDK YIT.....AK YDATTAIEAK ALNKEALQAE AGLPVDRKIP LIAFIGRLEE QKGPDVMAAA IPELM.QEDV
rice/SSS     INDWNPSTDK FLP.....YH YSVDDL.SGK AKCKAELQKE LGLPIRPDVP LIGFIGRLDY QKGIDLIKLA IPDLM.RDNI

sweet potato QILILGTGKK KFEQQIEQLE VMYPDKARGV AKFNVPLAHM ITAGADFMLI PSRFEPCGLI QLHAMRYGTP CICASTGGLV
cassava      QIVILGTGKK KFEKQIEHLE VLYPDKARGV AKFNVPLAHM ITAGADFMLV PSRFEPCGLI QLHAMRYGTV PIVASTGGLV
pea          QIVVLGTGKK IMEKQIEVLE EKYPGKAIGI TKFNSPLAHK IIAGADFIVI PSRFEPCGLV QLHAMPYGTV PIVSSTGGLV
barly        QIILLGTGKK KFEKLLKSME EKFPGKVRAV VRFNAPLAHQ MMAGADLLAV TSRFEPCGLI QLQGMRYGTP CVCASTGGLV
wheat        QIVLLGTGKK KFERLLKSVE EKFPTKVRAV VRFNAPLAHQ MMAGADVLAV TSRFEPCGLI QLQGMRYGTP CACASTGGLV
rice/GBSS    QIVLLGTGKK KFEKLLKSME EKYPGKVRAV VKFNAPLAHL IMAGADVLAV PSRFEPCGLI QLQGMRYGTP CACASTGGLV
rice/SSS     QFVMLGSGDP GFEGWMRSTE SGYRDKFRGW VGFSVPVSHR ITAGCDILLM PSRFEPCGLN QLYAMQYGTV PVVHGTGGLR
                                                                  BOX 2                          BOX 3
sweet potato DTVKEGYTGF HMGAFNVDCE TVDPEDVLKV ITTVGRALAI YGTLAFTEMI KNCMSQELSW KGPAKNWETV LLSLGVAGSE
cassava      DTVKEGYTGF QMGALHVECD KIDSADVAAI VKTVARALGT YATAALREMI LNCMAQDLSW KGPARMWEKM LLDLEVTGSE
pea          DTVKEGYTGF HAGPFDVECE DVDPDDVDKL AATVKRALKT YGTQAMKQII LNCMAQNFSW KKPAKLWEKA LLNLEVTGNV
barly        DTIVEGKTGF HMGRLSVDCN VVEPADVKKV ATTLKRAVKV VGTPAYQEMV KNCMIQDLSW KGPAKNWEDV LLELGVEGSE
wheat        DTIVEGKTGF HMGRLSVDCN VVEPADVKKV VTTLKRAVKV VGTPAYHEMV KNCMIQDLSW KGPAKNWEDV LLELGVEGSE
rice/GBSS    DTVIEGKTGF HMGRLSVDCK VVEPSDVKKV AATLKRAIKV VGTPAYEEMV RNCMNQDLSW KGPAKNWENV LLGLGVAGSA
rice/SSS     DTVENFNPFA EKG.EQGTGW AFSPLTIEK. .....NAVGI ..ADGNFDIQ G...TQVLLG GSNEARHVKR LYMGPCRLTV

sweet potato PGVEGEEIAP LAKENVATP*
cassava      PGTEGEEIAP LAKENVPTP*
pea          AGIDGDEIAP LAKENVATP*
barly        PGIVGEEIAP LAMENVAAP*
wheat        PGIVGEEIAP LALENVAAP*
rice/GBSS    PGIEGDEIAP LAKENVAAP*
rice/SSS     *..........  ..........
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Fig. 1. Amino acid sequence comparisons of sweet potato GBSSI with starch synthases of other plant species. Conserved regions are underlined and labeled as BOX 1, 2 and 3. Asterisk indicates the ADP-glucose binding site. Starch synthase sequences of other plants were obtained from Wisconsin Genetics Computer Group (GCG) Saftware Package version 9.0.

site, Lys-X-Gly-Gly (K-X-G-G). The Lys residue in *E. coli* glycogen synthase was identified to involve in ADP-glucose binding (Furukawa *et al.*, 1994). Glycogen is a D-glucose polymer similar to starch, but glycogen is a much highly branched glucan molecule than starch (Shewmaker *et al.*, 1994). Glycogen synthase catalyzed glucosyltransferring activity from ADP-glucose to non-reducing end of glucan molecules is a fashion similar to starch synthase (Kumar *et al.*, 1986). Although the K-X-G-G sequence was also identified in plant starch synthases, there is no direct evidence to show that this region is involved in the ADP-glucose or UDP-glucose binding.

Spss67 encoded an active starch synthase

In order to characterize the product of *spss67* clone, the *spss67* cDNA was constructed into a pET-21a expression vector and transferred into *E. coli* BL21 (DE3) strain. Transformants containing the *spss67* cDNA overexpressed a 67-kDa protein in the inclusion body fraction after IPTG induction (Fig. 2A). The protein encoded by *spss67* cDNA could be recognized by an antiserum raised against the GBSSI protein isolated from starch granules of potato (Fig. 2B). This protein product was tightly bound with the inclusion body of *E. coli*, and this phenomenon could not be improved by changing culture conditions. These conditions included decreases in the temperature of cultures or decreases in IPTG concentrations for inducing protein expressions. Furthermore, addition of 2.5 mM betaine /1 M sorbitol into the medium also failed to improve the situation (Blackwell and Horgan, 1991) (data not shown).

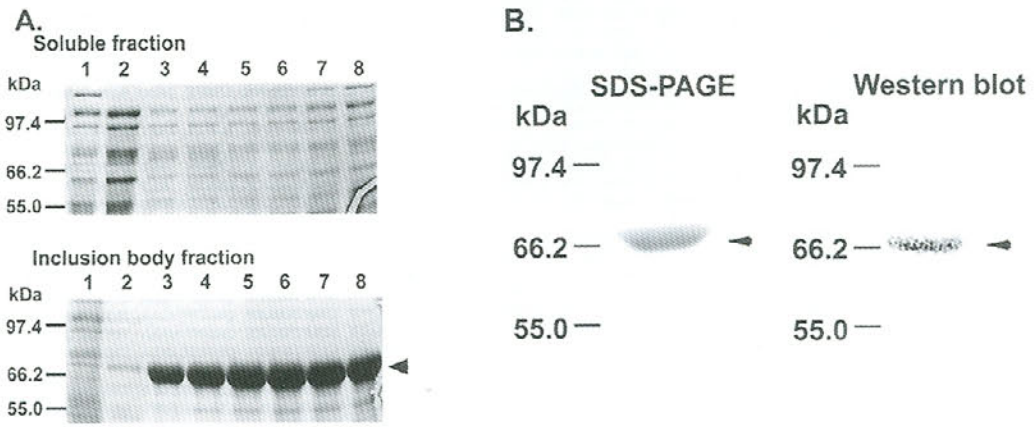

Fig. 2. Identification of *spss67* clone protein product expressed in an *E. coli* expression system. A. Proteins were isolated from soluble and inclusion body fractions of *E. coli*. Lane 1, native plasmid transformants. Lane 2 to 8, expressions of the *spss67* clone in transformants after IPTG induction for 1, 2, 3, 4, 5, 6 and 7 hrs, respectively. Proteins were stained with Coomassie Brilliant Blue on a 7.5% SDS-PAGE. B. Proteins extracted from inclusion body fractions of the *spss67* transformants after IPTG induction for 3 hrs were stained with Coomassie Brilliant Blue on a 7.5% SDS-PAGE (left panel) and immunoblotted with antiserum against the GBSSI of potato (right panel). Arrow indicates GBSSI.

While *spss67* cDNA encoded a 67-kDa protein, the mature GBSSI of sweet potato had a molecular mass of only 59 kDa (Wang *et al.*, 1999). The N-terminus of the mature GBSSI isolated from tuberous roots was identified as KQQGMNLVFVGCEEGP that corresponded to the sequence starting from the 78th amino acid of the protein product of *spss67* cDNA clone (Fig. 3A). This result suggests that sweet potato GBSSI protein contained a transit

peptide of 77 amino acid. This peptide showed a low identity of 27-47% with that of other species. The transit peptide of sweet potato GBSSI had hydrophobic regions in the initiation and end of the peptide (Fig. 3B), and the structure was also present on other bound form of starch synthases (Fig. 3B). These observations suggest that the hydropathic distributions of transit peptides might be more important than the amino acid sequence *per se* for functions.

Since GBSSI and SSS could be distinguished based on biochemical properties, the protein expressed in *E. coli* was analyzed for starch synthase activity using different primers, e.g., glycogen, amylopectin and amylose. When reactions were supplied with glycogen or amylopectin, the activity of starch synthase prepared from the *spss67* cDNA transformants was similar to that of native plasmid transformants, which contained only glycogen synthase (Figs. 4A and 4B). However, when the primer was replaced by amylose in reaction solutions, the activity of starch synthase isolated from *spss67* cDNA transformants increased along with the time of reactions, and the activity was significantly higher than that of the pET-21a vector transformants (Fig. 4C). In these experiments, the high baseline was caused by artifical binding of ^{14}C ADP-glucose, and the binding could not be washed completely. These results indicated that the protein product of *spss67* preferred to use amylose as a primer to polymerize the glucose molecules characteristic of the WAXY proteins.

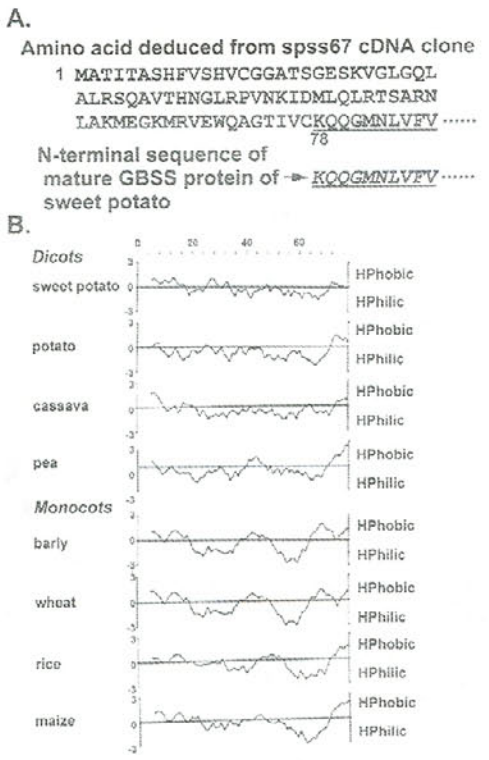

Fig. 3. Amino acid sequence and hydropathic profile of transit peptide of sweet potato GBSSI protein. A. Transit peptide sequence of GBSSI. B. Hydrophobicity plots of the N-terminal residues of GBSSI from four dicot and four monocot plants. These were predicted by Wisconsin Genetics Computer Group (GCG) Saftware Package version 9.0.

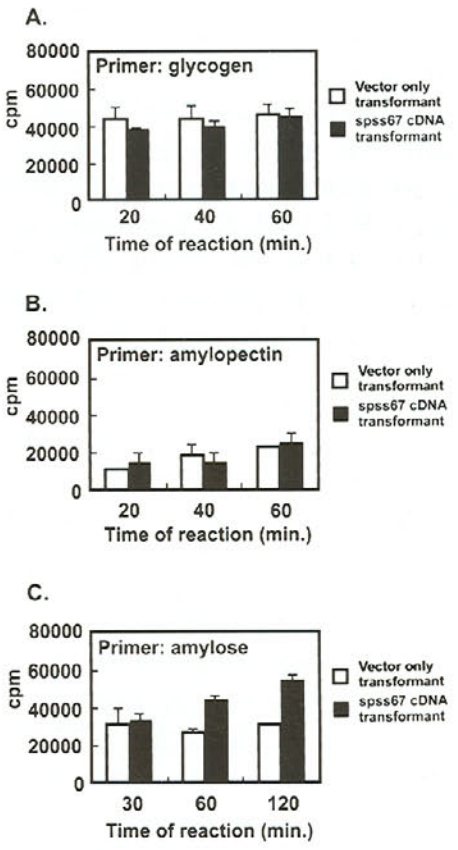

Fig. 4. Activity assay of sweet potato GBSSI expressed in *E. coli*. Starch synthase activity was assayed using ADP glucose-^{14}C as a substrate under different priming conditions (A. glycogen, B. amylopectin or C. amylose primer). Radioactivity was determined at the indicated time.

In conclusion, a sweet potato starch synthase gene (*spss67* cDNA clone) was isolated from tuberous roots library, and the deduced amino acid sequence shared a high degree of homology with that of GBSS prepared from other species, but was quite different from SSS. This cDNA clone encoded a 67-kDa protein when expressed in an *E. coli* expression system, and this protein product could be recognized by anti-potato GBSSI antibody. The starch synthase encoded by *spss67* cDNA preferred to use amylose as a primer for glucose polymerization, and the activity of this protein was low when the amylose primer was replaced by amylopectin or glycogen in the reaction mixture. Therefore, those studies provide a strong evidence to indicate that the *spss67* cDNA clone was a bound form of starch synthase gene but not the SSS.

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利用大腸桿菌表現甘藷澱粉粒結合性澱粉合成酵素及其特性分析

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摘　　要

本研究的主要目的是利用大腸桿菌(*E. coli*)表現甘藷之澱粉合成酵素基因(*spss67*)，並藉由氨基酸序列比對、抗體辨認及生化特性分析鑑定此澱粉合成酵素基因究竟是屬於澱粉粒結合性澱粉合成酵素(granule-bound starch synthase; GBSS)或是可溶性澱粉合成酵素(soluble starch synthase; SSS) 基因。西方雜合分析結果顯示由 *E. coli* 表現的 *spss67* 蛋白質產物可被馬鈴薯 GBSSI 抗體辨認。經氨基酸序列比對顯示此蛋白與其他植物之 GBSSI 有較高的同源性，而與 SSS 的相似性則較低。此外，經 N 端氨基酸序列分析，得知甘藷之 GBSSI 蛋白具有一段 77 個氨基酸的導引訊息 (transit peptide)。澱粉合成酵素活性分析的結果顯示 *spss67* cDNA 之蛋白產物需以直鏈澱粉 (amylose) 做為做為引子以進行葡萄糖聚合，但其無法以支鏈澱粉 (amylopectin) 及肝醣 (glycogen) 做為引子以進行反應，此結果提供一直接的證據證明 *spss67* cDNA 為甘藷之 GBSSI 基因。

關鍵詞： cDNA，澱粉合成酵素，直鏈澱粉，支鏈澱粉，導引訊息，轉型菌株。

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