台灣農業化學與食品科學（中華民國九十六年四月／第四十五卷第二期：第九十一至一〇〇頁）
Taiwanese Journal of Agricultural Chemistry and Food Science (April, 2007) 45(2): 91-100

Cloning and Expression of Sweet Potato Tuber Sucrose Phosphate Synthase Gene in *Escherichia coli*

Wei-Liang Chen, Chien-Chih Yang and Ping-Du Lee*

Department of Biochemical Science and Technology; Institute of Microbiology and Biochemistry, National Taiwan University, Taipei 106, Taiwan

(Accepted for publication: March 5, 2007)

The cDNA coding for sucrose phosphate synthase (SPS) from tuber of sweet potato has been cloned, sequenced and expressed in *Escherichia coli* BL21 (DE3). The cDNA coded for 1048 amino acids and was with predicted molecular mass of 116 *kDa*. The deduced amino acid sequences showed 66 and 80% identity with the SPSs from spinach and potato, respectively, and possessed motifs for the phosphorylation site which may associate with light- and osmotic stress-induced regulation. The SPS gene was subcloned into pET21b vector and subjected to heterologous expression in *E. coli* BL21 (DE3). Purification of expressed SPS from *E. coli* employed His-tagged affinity column and FPLC Mono-Q anion-exchange chromatography. The purified protein was active and showed a clear major band close to the expected molecular mass of 116 *kDa*. The expressed protein from *E. coli* was not allosterically regulated by Glc 6P and Pi, and the K_m for the substrates UDPGlc and Fru 6-P were 21.3 and 7.3 *mM*, respectively. The expressed protein displayed a specific activity of 0.92 *μmol* per min per mg protein, and an optium pH of 7.5. The enzyme was activated by Mg^{2+}, Ca^{2+} and Mn^{2+}. The nucleotides, UDP, UTP inhibited the enzyme activity about 30-40%, and the sugar and sugar phosphates have little or no effect on its activity.

Key words: Sweet potato, cDNA cloning, Gene expression, Sucrose phosphate synthase, *Escherichia coli*, *Ipomoea batatas*.

甘藷塊根蔗糖磷酯合成酶基因在大腸桿菌中之表現

陳 韋 良　楊 健 志　李 平 篤*

國立臺灣大學生化科技學系
微生物與生化學研究所

（接受刊載日期：中華民國九十六年三月五日）

選殖自甘藷塊根之蔗糖磷酯合成酶(SPS)基因，經大腸桿菌系統加以表現。其 cDNA 經表現後可轉譯出 1048 個胺基酸，分子量為 116 *kDa*。所推導出的胺酸序列，與菠菜和馬鈴薯者分別有 66 及 80%同質性；且具有可進行磷酸化之可能區位，用以表現對光照和滲透逆境之調控。SPS 基因經與質體 pET21b 重組次選殖後，轉型於大腸桿菌 (*Escherichia coli*) BL21 (DE3)菌株中加以表現。其表現出分子量在 116 *kDa* 之蛋白質，以 His-tagged 親和層析管柱及 FPLC Mono-Q 陰離子交換層析法純化後，測定活性並分析其性質得知：Glc 6P 和 Pi 不具有異位調控；對基質 UDPGlc 和 Fru 6P 之 K_m 值分別為 21.3 和 7.3 *mM*；其酵素比活性為 0.92 *μmol/min/mg* protein；而反應之最適 pH 為 7.5；表現之酵素會受 Mg^{2+}, Ca^{2+}和 Mn^{2+}活化；核苷酸 UDP 及 UTP 則可抑制 30-40%活性；而糖及糖磷酯對表現之 SPS 活性影響不大。

關鍵字：甘藷，cDNA 選殖，基因表現，蔗糖磷酯合成酶，大腸桿菌。

INTRODUCTION

In most plants, sucrose is the major export form of photosynthates from the source tissue to the sink tissue, and it also plays an important role as regulator for gene expression of photosynthetic[1] and non-photosynthetic[2] organisms. Sucrose phosphate

* Corresponding author. E-mail: pingdu@ntu.edu.tw

synthase (SPS; EC 2.4.1.14), plays a central role in sucrose synthesis pathway, which catalyzes the following reaction:

Fructose 6-phosphate + UDPglucose ↔ Sucrose 6'-phosphate + UDP

In source tissue leaves, SPS is regulated by the binding of Glc 6P and Pi at an allosteric site[3], and by covalent modification through a mechanism involving protein phosphorylation/dephosphorylation[4]. In the leaves of some plants, SPS activity often increases upon illumination and decreases in the dark. In spinach[4] and maize[5], light/dark regulation is achieved by reversible protein phosphorylation at Ser_{158}[6], and Ser_{162}, respectively[7]. A second regulatory phosphorylation site has been identified in spinach SPS at Ser_{424}, which is involved in osmotic stress-induced activation of the enzyme[8].

Sucrose phosphate synthase is also present in sink tissues, which may contribute to several important processes including synthesis and export of sucrose in germinating seeds and sprouting tubers[9]. In sink tissues, the role of SPS is less well studied. Nevertheless, similar conserved regulation mechanisms are proposed. In potato tubers, protein phosphorylation has been suggested to regulate SPS activity in an analogous way to the SPS activity from leaves[10].

The full-length cDNA encoding SPS from cyanobacteria has been isolated, and expressed in *Escherichia coli*. Its molecular mass is predicted to be 81.5 *kDa*, which is smaller than those 115-120 *kDa* homologs from the higher plant. Unlike the higher plant, ADPGlc, CDPGlc, and GDPGlc can substitute for UDPGlc as the glucosyl donor for the SPS from cyanobacteria, and the enzyme was not activated by Glc 6P, and only weakly inhibited by Pi[11].

Here we report the isolation of the SPS cDNA from sweet potato tuber by RT-PCR. The complete SPS cDNA has been sequenced and compared with those from other species. And polypeptide deduced from the sweet potato tuber SPS has been predicted, and also compared with potato tuber and spinach leaf. The expressed SPS was subjected to characterization in the regulation and kinetic studies.

MATERIALS AND METHODS

Materials

Host strain *Escherichia coli* BL21 (DE3) and pET21b vector were purchased from Novagen (Madison, WI. USA). Primers were synthesized by Life Technologies (Gibco BRL, San Diego, USA). Biochemical reagents were obtained from Sigma-Aldrich (St. Louis, USA). Restriction endonucleases and DNA modifying enzymes were obtained from New England Biolabs (Boston, USA).

Plant materials

The sweet potato, *Ipomoea batatas* (L.) Lam. cv. Tainong 57, was grown in the University farm, and fresh young roots were cut immediately before use.

Cloning and sequencing

Total RNA was isolated from sweet potato tuber by TRIZOL® Reagent (Invitrogen, Carlsbad, CA, USA). Poly(A)$^+$ RNA was purified from total RNA with a PolyAtract mRNA isolation system (Promega, Wisconsin, USA). First strand cDNA was synthesized from Poly(A)$^+$ RNA using a First strand cDNA synthesis Kit (Amersham Pharmacia Biotech, Piscataway, NJ. USA). To amplify the full-length cDNA (GenBank accession number AF439861 for Wei-Liang Chen; Jong-Ching Su and Ping-Du Lee) encoding sweet potato tuber SPS together with suitable restriction enzyme sites, two primers, the forward (5'-CATATGGCAGGAAATGATTGGAAAA CAGT-3') and the reverse (5'-CTCGAGATATCCTTTGAGTACC-GCTAGC-3'), were designed. The condition for PCR amplification were as follows : one cycle of 1 *min* at 94℃; 30 cycles of 94℃/1 *min*, 55℃/1 *min* and 72℃/5 *min*. In a PCR system 2400 thermocycler (Applied Biosystems, Foster City, CA. USA). The PCR product was purified by GFX™ PCR DNA and Gel Band Purification Kit

(Amersham Pharmacia Biotech, Piscataway, NJ. USA). The purified DNA fragment was digested with *Nde* I and *Xho* I, and cloned into pET21b expression vector (Novagen, Madison, WI. USA). The ligation products were transformed into *E. coli* strain JM109 by electroporation (MicroPluser™, Bio-Rad Laboratories, Inc. Hercules, CA. USA). The recombinant pET21b/SPS was confirmed by restriction analysis and double- strand DNA sequencing.

Protein expression and purification

Escherichia coli BL21(DE3) cells harboring pET21b/SPS were grown in LB medium containing 100 *μg/mL* ampicillin overnight at 37℃ for small scale culture. The overnight culture (20 *mL*) was transferred to 1 L of the LB medium which contained 1 *mM* PMSF. The cells were grown in a rotatory shaker at 37℃ until the cell density reached an A_{600} of 0.6, and then IPTG was added to a final concentration of 0.4 *mM*. After incubation at 37℃ for 18 *h*, the cells were harvested by centrifugation at 4,000 *g* for 20 *min* under 4℃ and the pellet was stored at －80℃. To purify the His_6-tagged fusion protein under native condition, the cell pellet was resuspened in 40 *mL* of lysis buffer (25 *mM* Hepes-NaOH, pH 7.5, 500 *mM* NaCl, 1 *mM* imidazole, 1 *mM* PMSF) and then lysed by sonication. The crude lysate was centrifuged at 8,000 *g* for 10 *min* at 4℃. The supernatant was mixed gently with 1 *mL* of Ni-NTA resin by shaking (200 *rpm* on a rotary shaker) at 4℃ for 60 *min*. The lysate-Ni-NTA mixture was loaded into a column and washed with wash buffer that containing 20 *mM* imidazole. The bound proteins were eluted with elution buffer containing 50 *mM* imidazole. The SPS fractions were collected and concentrated by a Centricon YM-100 centrifugal filter device with a cut off at Mr 100,000 (Millipore,), and exchanged into buffer A (50 *mM* Hepes-NaOH, pH 7.5, 15 *mM* $MgCl_2$, 1 *mM* DTT, 1 *mM* EDTA). The concentrated SPS was then applied to a Mono-Q column (HR 5/5; Amersham Pharmacia Biotech.) which has been preequilibrated with 20 *mL* buffer B (50 *mM* Hepes-NaOH, pH 7.5, 15 *mM* $MgCl_2$, 1 *mM* EDTA, 1 *mM* DTT, 10% glycerol). The Mono-Q column was eluted using a fast protein liquid chromatography (FPLC) system (Amersham Pharmacia Biotech.) at a flow rate of 1 *mL* per *min*, with a gradient from 0-1 *M* NaCl in buffer B for 68 *min*. The fractions (1 *mL* each) were collected and assayed for SPS activity.

Assay of SPS

SPS activity was monitored by the anthrone test[4]. In this test, 45 *μL* extract of protein sample was made to a final volume of 70 *μL* containing 10 *mM* Fru 6P, 10 *mM* UDPGlc, 50 *mM* Hepes-NaOH, pH 7.5, 15 *mM* $MgCl_2$, 1 *mM* EDTA, 1 *mM* DTT, and incubated for 15 *min* at 25℃. The reaction was stopped by adding 70 *μL* 30% (*w/v*) KOH and heated to 95℃ for 5 *min*, followed by cooling at 4℃. Then 1 *mL* 0.14% (*w/v*) anthrone in 95% H_2SO_4 was added. After incubation for 20 *min* at 37℃, the absorbance at wavelength of 620 *nm* was measured. One unit was defined as the amount of enzyme required for producing 1 *μmol* of sucrose from UDPGlc and Fru 6P at 25℃ and pH 7.5 per *min*.

Gel electrophoresis and protein determination

10% SDS-PAGE was carried out as described by Laemmli[12]. The samples were treated by heating at 95℃ for 5 *min* in sample loading buffer (80 *mM* Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 2% β-mercaptoethanol, 0.02% bromo-phenol blue) before loading into the gels. After running, the gel was stained with Coomassie Blue R250 (Sigma-Aldrich, St. Louis, MO. USA). Protein concentration was measured as described by Bradford[13] with bovine serum albumin as reference.

RESULTS

Comparison of the SPS sequences from sweet potato and other species

Deduced amino acid sequences of sweet

potato tuber SPS at the N-terminus and the phosphorylation site motifs that associated with light- and osmotic stress-induced regulation of SPS in many plants were compared in Figure. 1. Sweet potato tuber SPS had the regulatory phosphorylation site and the corresponding serine residue and its proximal sequence was well conserved. Another regulatory phosphorylation site, Ser_{424} in spinach SPS, has been identified as an osmotic-stress site[8]. When comparing with spinach SPS, the regulatory phosphorylation sites in sweet potato tuber SPS was at Ser_{150} and Ser_{416}, respectively.

Construction of His_6-tagged SPS expression vector

It was found that SPSs are highly conserved in both N- and C-terminus[10]. Therefore, it is quite possible that the PCR product amplified from sweet potato using primers designed according to those conserved species would produce full-length cDNA. The SPS cDNA from sweet potato was amplified by RT-PCR and isolated. The reaction yielded a single cDNA product with the size of 3147 *bp*, was inserted into expression vector pET21b at restriction enzymes *Nde* I and *Xho* I sites. Figure 2 shows the construction strategy of plasmid pET21b/SPS for the expression of His_6-tagged SPS protein. This vector is designed to express a C-terminal His_6-tagged fusion protein under the control of the IPTG-inducible T7 promoter. In this case, the His_6-tagged SPS gene is placed in the correct reading frame with the initiation codon (ATG) supplied by pET21b vector. The initiation codon of the His_6-tagged SPS gene is within 7 *bp* from the ribosome-binding site. A strong T7 transcriptional terminator is placed downstream of the gene (Figure 2).

Expression of the recombinant His_6-tagged SPS gene in *E. coli* BL21 (DE3) cells

Escherichia coli BL21 (DE3) cells haboring the recombinant plasmid was grown to early-log phase and induced by the addition of IPTG. The medium which contained 1 *mM* PMSF could prevent protein degradation and maintain the activity of SPS (Figure 3). Heterologous

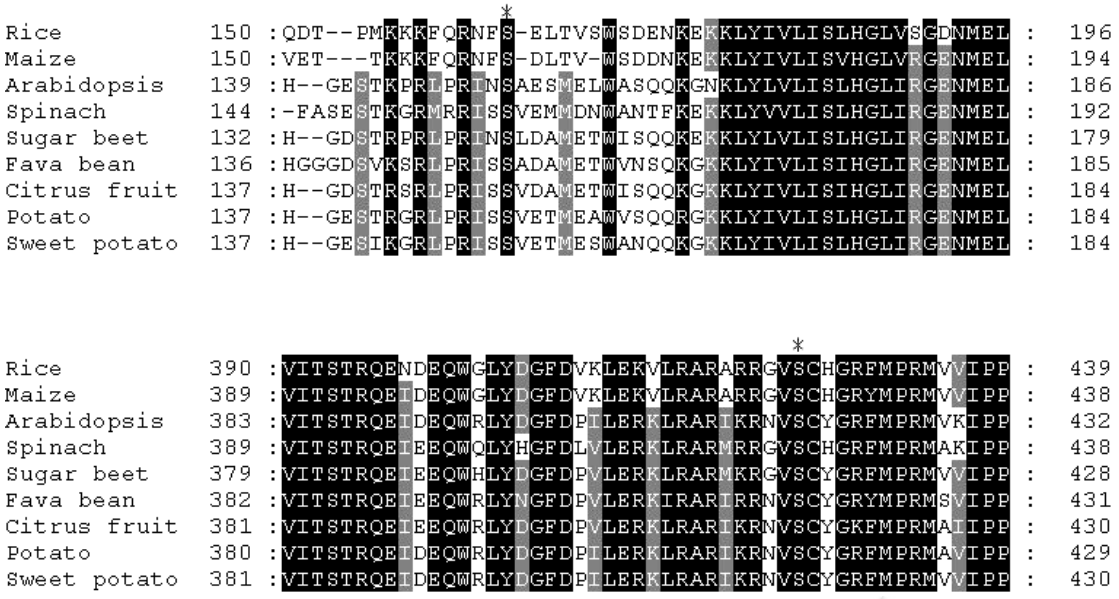

Fig. 1. Alignment of the deduced amino acid sequence of SPS from sweet potato tuber and from other sources in the region of the regulatory phosphorylation sites. The GenBank accession numbers of the sequence are: maize (Zea mays), M97550 [23]; rice (*Oryza sativa*), U33175 [21]; potato (*Solanum tuberosum*), X73477 [14]; sweet potato (*Ipomoea batatas*), AF439861(this article); fava bean (*Vicia faba*), Z56278 [17]; citrus fruit (*Citrus unshiu*), O22060 [15]; Arabidopsis (*Craterostigma plantagineum*), Y11795 [19]; sugar beet (*Beta vulgaris*), X81975 [16]; spinach (*Spinacia oleracea*), L04803 [24]. * indicated the regulatory phosphorylation sites.

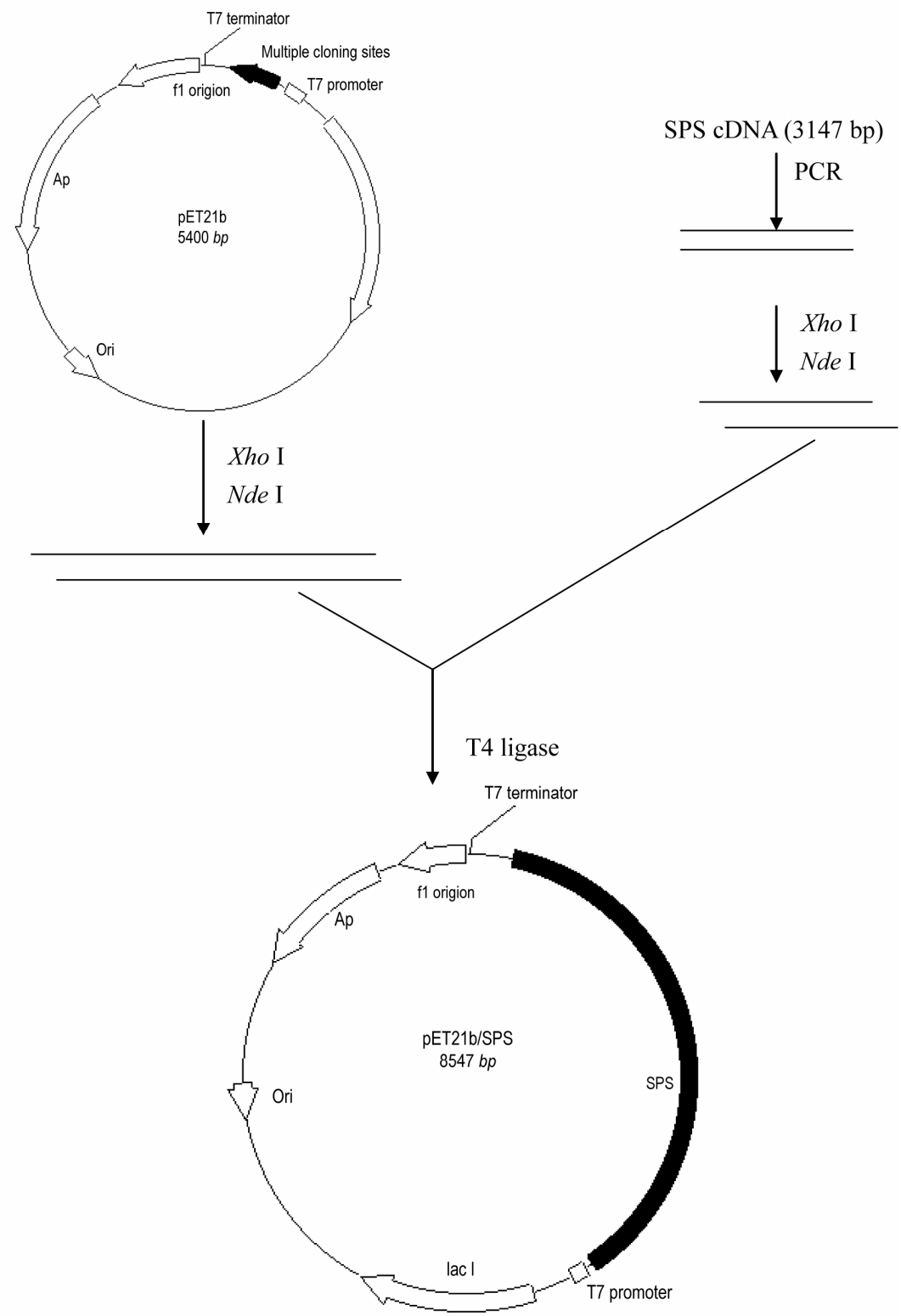

Fig. 2. Construction of recombinant expression plasmid pET21b/SPS. The SPS gene was inserted into *Nde* I and *Xho* I sites of expression vector pET21b.

protein expression of the recombinant His_6-tagged SPS gene in *E. coli* was analyzed by 10% SDS-PAGE. The optimum condition was determined to be induced by 0.4 *mM* IPTG for 18 *h*. The crude extracts from induced cultures displayed SPS activity of 0.09-0.114 *μmol* per min per mg protein, whereas extracts from cells containing the pET21b vector alone showed no SPS activity.

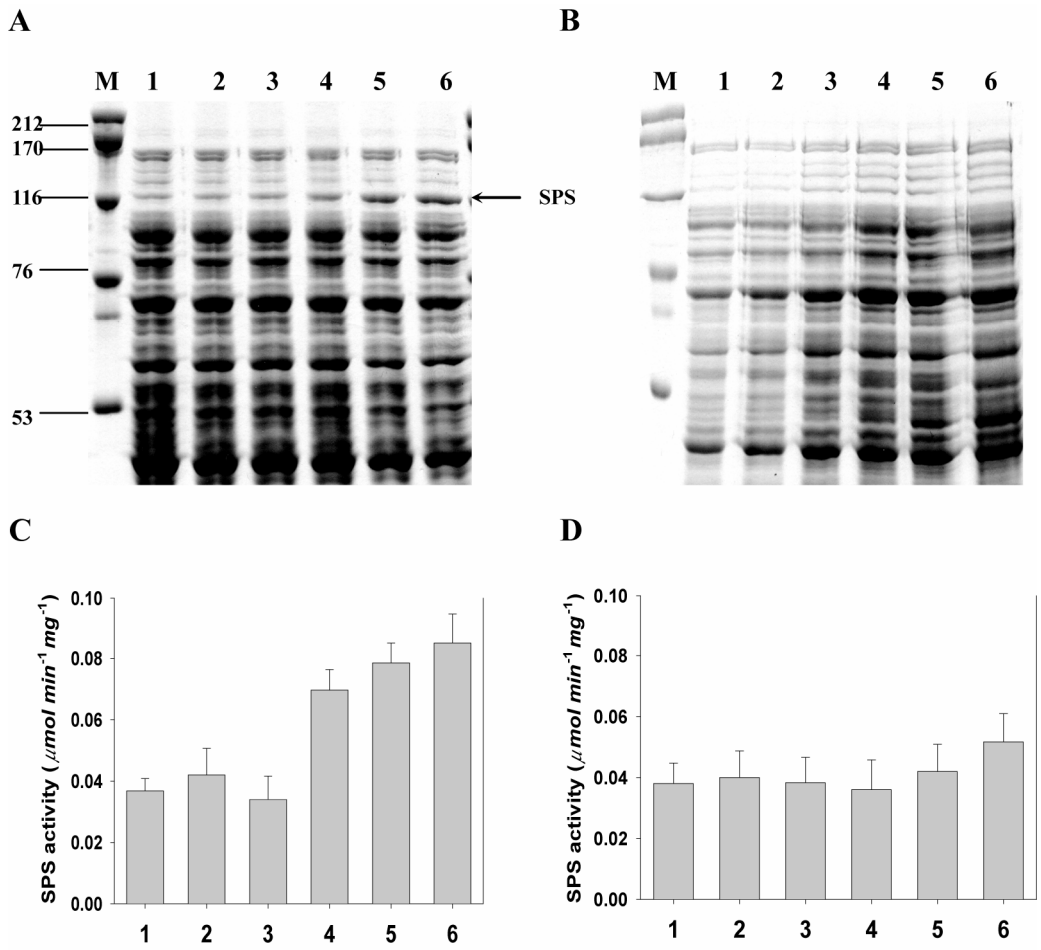

Fig. 3. Protein expression of pET21b/SPS in *E. coli* was analyzed on 10% SDS-PAGE. LB medium with (A) or without (B) 1 *mM* PMSF at different concentration of IPTG induction and cells were induction at 37℃ for 3 *h*. C and D, His_6-tagged SPS activity in crude extract from cultures with different IPTG induction (C, the same samples as A; D, the same samples as B). Lane 1, total protein without IPTG induction; lane 2, total protein from cultures with 0.0016 *mM* IPTG induction; lane 3, total protein from cultures with 0.0063 *mM* IPTG induction; lane 4, total protein from cultures with 0.025 *mM* IPTG induction; lane 5, total protein from cultures with 0.1 *mM* IPTG induction; lane 6, total protein from cultures with 0.4 *mM* IPTG induction. M: protein molecular mass markers. The gel was stained with Coomassie brilliant blue R250.

Purification and characterization of SPS

The purification procedure based on 1 *L* of the culture was summarized in Table 1. His_6-tagged SPS was first purified by Ni^{2+} chelating affinity chromatography (Figure 4, lane 3). The protein fraction with His_6-tagged affinity was loaded on a Mono-Q column using a continuous NaCl gradient. SPS was eluted as a single peak between 270 and 300 *mM* NaCl. A single band with a molecular mass about 116 *kDa* appeared in SDS-PAGE (Figure 4, lane 4). Purification by Mono-Q column resulted in over 95% pure SPS as estimated from CBR staining gel. About 3.9 mg homogeneous protein was obtained from 1 *L* culture with a recovery of approximate 17%. The purified His_6-tagged SPS showed activity of 0.92 *μmol* per min per mg protein (Table 1). The enzyme was not allosterically regulated by Glc 6P and Pi (Figure 5). The pH optimum was about 7.5. At a pH range of 6 to 8, His_6-tagged SPS had 70% of the maximum activity (Figure 6). The dependence of the initial velocity of the enzyme reaction upon substrate concentration was studied in varied concentrations of the substrates, UDPGlc or Fru6P. His_6-tagged SPS showed hyperbolic substrate saturation kinetics with UDPGlc and Fru 6P. The K_m values for UDPGlc and Fru 6P were calculated as 21.3 and 7.3 *mM*, respectively

Table 1. Purification of sweet potato tuber SPS expressed in *E. coli*.

Purification step	Volume (*mL*)	Protein (*mg*)	Total activity (*units*)[1]	Specific activity (*U/mg*)	Yield (*%*)	Purification (fold)
Crude lysate	40	188	20.6	0.11	100	1.0
Ni^{2+}-NTA	6	12.1	6.8	0.56	33	5.1
FPLC Mono Q column	3	3.9	3.6	0.92	17.4	8.4

1 One unit is defined as the amount of enzyme required for producing 1 *μmol* of sucrose from UDPGlc and Fru 6P at 25 ℃ and pH 7.5 per *min*.

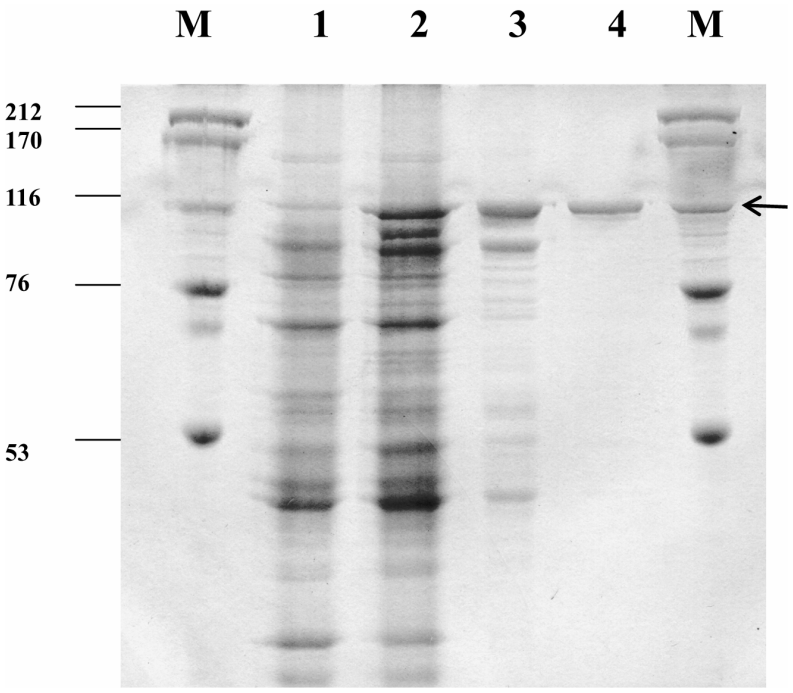

Fig. 4. 10% SDS-PAGE analysis of His_6-tagged SPS from different purification steps. The gel was stained with Coomassie brilliant blue R250. Lane 1, total protein extract without IPTG induction; lane 2, total protein extract after IPTG induction; lane 3, 50 *mM* imidazole elution; lane 4, Mono Q fraction. M: protein molecular mass markers. ← indicates the band of expressed SPS.

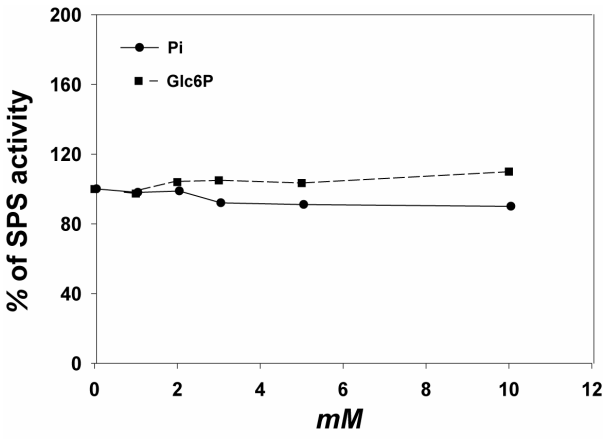

Fig. 5. Effect of different Pi and Glc 6P concentrations on the activity of expressed SPS from *E. coli*.

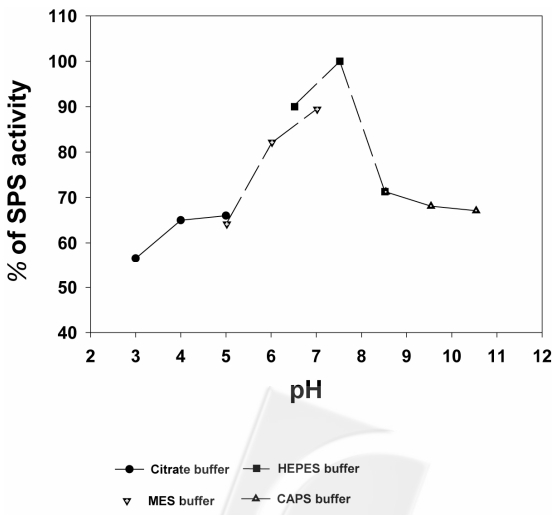

Fig. 6. Effect of various *p*H values on the activity of expressed SPS from *E. coli*.

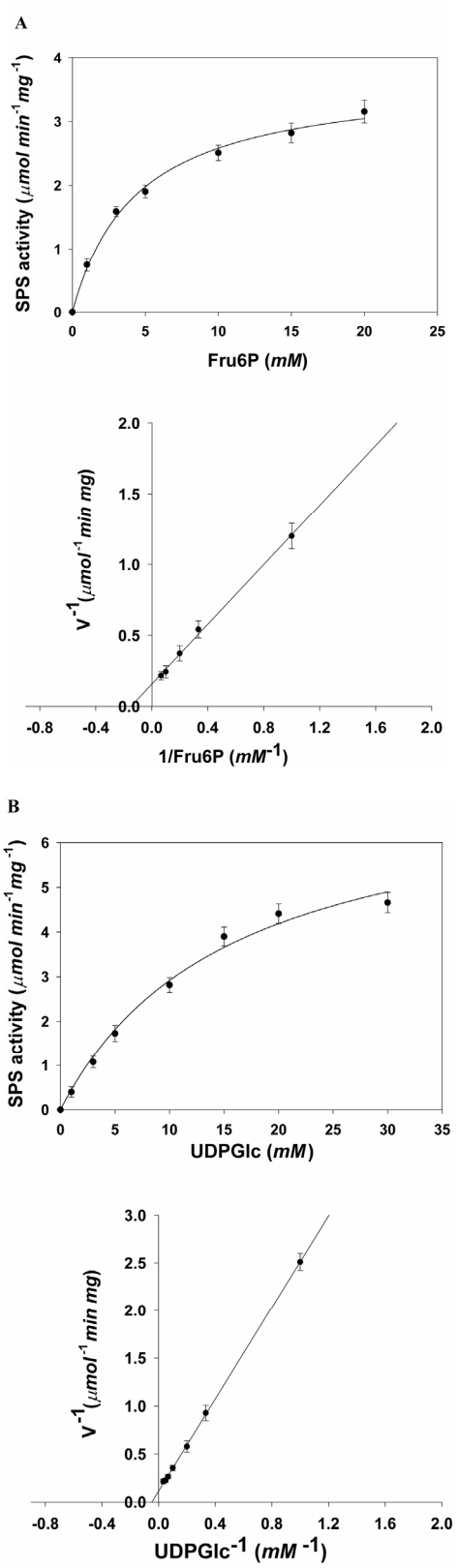

Fig. 7. Effect of different Fru 6P (A) and UDPGlc (B) concentrations on the activities of expressed SPS. Both hyperbolic graph (upper) and double reciprocal plots (bottom) are shown of SPS. The lower panels were the double reciprocal plots from the upper graphs.

(Figure 7). The influence of various nucleoside mono-, di-, and triphosphates on enzyme activity was summarized in Table 2. It showed that ATP, CTP, ADP and AMP had no effect, GTP caused slight inhibition, and UTP and UDP inhibited enzyme activities up to 30-40%. The effects of divalent cations were studied. It was found that the enzyme activity could increase up to 140% by 20 *mM* $MgCl_2$, and slightly be activated by $MnCl_2$ and $CaCl_2$. The sugar and sugar phosphates had little or no effect on its activity (Table 2).

Table 2. The effect of nucleotides, sugar, sugar phosphates and divalent metal ions on the activity of sweet potato tuber SPS expressed in *E. coli*.

Addition	SPS activity (% of control)
ATP	104
ADP	106
AMP	108
GTP	82
UTP	73
UDP	62
CTP	93
NAD	93
FBP	83
Glc1P	103
Glc	93
$MnCl_2$	129
$CoCl_2$	99
$CaCl_2$	120
$MgCl_2$	144

1 All nucleotides, sugar and sugar phosphates added at a final concentration of 10 *mM*.

2 Divalent metal ions added at a final concentration of 20 *mM*.

DISCUSSION

The putative ORF of the sweet potato tuber SPS gene was 3147 *bp* and encoded 1048 amino acids, which shows high homology with other SPS in higher plants. Due to the recombinant protein is functional, we believed that this clone contained the full-length cDNA of SPS. The deduced amino acid sequence analysis revealed 80% sequence identity with potato[14], 76% with citrus fruit[15], 70% with sugar beet[16], 72% with fava bean[17], 66% with spinach[18],

65% with Arabidopsis[19], 53% with maize[20], and 51% with rice[21]. These comparison also showed that the sweet potato tuber SPS sequence was highly homologous to the SPSs from dicotyledons and less similar to those from monocotyledons plant. Deduced amino acid sequences of sweet potato tuber SPS has a molecular mass about 116 *kDa*, resembling that in spinach[18], maize[20], and sugar beet[16]. The sequence comparison revealed a high homology at the regulatory phosphorylation sites. SPS has been shown containing phosphorylation at seryl residues *in vivo*[10]. The regulatory phosphorylation motif targeted by SPS kinase contains the consensus motif B-Hy-X-B-X2-Ser, where Ser represents the phosphate acceptor, X is any amino acid, Hy is a hydrophobic residue, B is a basic residue[7]. In the current study on sweet potato tuber SPS, the Ser_{150} residue and its proximal sequence were well conserved and considered to be the counterparts corresponding to Ser_{158} in spinach[4] and Ser_{162} in maize leaves[6]; The other regulatory phosphorylation site is Ser_{424} in spinach, had been identified as osmotic-stress site, and phosphorylation of Ser_{424} may partially antagonize the inhibitory effect of phosphorylation of Ser_{158} (in the selective assay)[8].

During the trials of expression of sweet potato tuber SPS in *E. coli*, the lack of PMSF in medium led to protein degradation and low activity. In contrast, the inclusion of 1 *mM* PMSF in LB medium prevented protein degradation and maintained the activity of SPS. A summary of the purification of *E. coli* expressed SPS is shown in Table 1. The first step employed Ni^{2+} chelating affinity chromatography, resulting in many bands on SDS-PAGE. The non-specific binding of the eluted contaminating proteins may be resulted from partially expressed SPS during induction or the His-tag is not properly extended in 3D space. A further purification on Mono Q column resulted in a pure preparation of His_6-tagged SPS and 17% of total activity from initial crude extract. The preparation after elution from Mono Q column yielded a major protein peak with enzyme activity corresponding to a molecular mass about 116 *kDa* on SDS-PAGE. This value is very close to the predicted molecular mass of sweet potato tuber SPS[22].

The *E. coli* expressed SPS would be affected by UTP and UDP, indicated that these nucleotides may play act as competitive inhibitors toward the UDPGlc-binding site of the enzyme. When comparing the kinetics studies of the *E. coli* expressed SPS (Figure 7) with that of the sweet potato tuber SPS[22], we found that the substrate saturation profiles for UDPGlc and Fru 6P are hyperbolic and they both show similar kinetic properties toward UDPGlc and Fru 6P[3]. In this paper, the K_m for Fru 6P of *E. coli* expressed SPS was 7.3 *mM* which is higher than that of the tuber extract, the K_m for UDPGlc was 21.3 *mM*, which is lower than that of tuber SPS, and the specific activity of expressed enzyme was 0.92 *U/mg* which is two fold higher than that of the native enzyme (Table 3). It is possible that the expressed SPS could be lacking of post-translational modification process. Moreover they were both activated by Mg^{2+}, Ca^{2+}, Mn^{2+} and their biological properties were similar[3,22]. SPS was known to be classified into three types of modulation *in vivo*[7]. Some species of higher plants exhibit a marked light activation on SPS (class I and class II), whereas others do not (class III). Class III SPS appears to be weakly regulated by metabolites (Glc 6P and Pi), and there is no evidence for covalent modification *in vivo* in response to short-term light/dark signals. Since the SPS from *E. coli* expressed and tuber extract were weakly regulated by Glc 6P and Pi, they belong to class III. In contrast, such an effect was not observed from the SPS of photosynthetic

Table 3. Kinetic constants and specific activity of SPS from sweet potato tuber and *E. coli* expressed

Origin	Optimum pH	Km (*mM*)		Specific activity (*U/mg*)
		UDPGlc	Fru 6P	
Tuber SPS[1]	7.5	31.2	5.3	0.41
Expressed SPS	7.5	21.3	7.3	0.92

1 Data were obtained from 500 *g* fresh sweet potato tuber[22]

tissues. The reason for the lack of such modulation is not clear. Up to now, it is still not well understood about the function and regulation of SPS in sink tissues such as in plant tuber and seeds, therefore, further studies are required to identify the allosteric binding site and the possibility of covalent modification of SPS. By using the *E. coli* expression system, we can produce pure active SPS without modification, which would be used to investigate the regulation of SPS *in vitro*, and it is necessary to purify SPS-kinase and SPS-phosphatase to investigate the effectors on their enzymatic properties.

ACKNOWLEDGEMENTS

Financial support for this study from the National Science Council of the Republic of China (Taiwan) (NSC93-2311-B-002-029) is gratefully acknowledged.

REFERENCES

(1) J. C. Jang and J. Sheen: Sugar sensing in higher plants. *Plant Cell*, **6**: 1665-1679 (1994).
(2) K. Nakamura, M. Ohto, N. Yoshida and K. Nakamura: Sucrose induced accumulation of β-amylase occurs concomitant with the accumulation of starch and sporamin in leaf-petiole cuttings of sweet potato. *Plant Physiol*., **96**: 902-909 (1991).
(3) C. D. Doehlert and S. C. Huber: Regulation of spinach leaf sucrose phosphate synthase by glucose-6-phosphate, inorganic phosphate, and pH. *Plant Physiol*., **73**: 989-994 (1983).
(4) J. L. Huber, S. C. Huber and T. H. Nielsen: Protein phosphorylation as a mechanism for regulation of spinach leaf sucrose-phosphate synthase activity. *Arch. Biochem. Biophy*., **270**: 681-690 (1989).
(5) S. C. Huber, J. and L. Huber: Regulation of maize leaf sucrose phosphate synthase by protein phosphorylation. *Plant Cell Physiol*., **32**: 319-326 (1991).
(6) R. W. J. McMichael, R. R. Klein, M. E. Salvucci and S. C. Huber: Identification of the major regulation phosphorylation site in sucrose phosphate synthase. *Arch. Biochem. Biophys*., **307**: 248-252 (1993).
(7) S. C. Huber and J. L. Huber: Role and regulation of sucrose-phosphate synthase in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., **17**: 431-444 (1996).
(8) D. Toroser, and S. C. Huber, Protein phosphorylation as a mechanism for osmotic-stress activation of sucrose phosphate synthase in spinach leaves. *Plant Physiol*., **114**: 947-955 (1997).
(9) J. S. Hawaker: Sucrose. In: *The Biochemistry of Storage Carbohydrate in Green Plants* (P. M. Dey and R. A. Dixon eds.), pp. 1-15. Academic Press, New York, USA (1985).
(10) R. Reimholz, P. Geigenberger and M. Stitt: Sucrose phosphate synthase is regulated via metabolites and protein phosphorylation in potato tubers, in a manner analogous to the enzyme in leaves. *Planta.*, **192**: 480-488 (1994).
(11) J. E. Lunn, G. D. Price and R.T. Furbank: Cloning and expression of prokaryotic sucrose-phosphate synthase gene from the cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Mol. Biol*., **40**: 297-305 (1999).
(12) U. K. Laemmli: Cleavage of structural protein during assembly of bacteriophage T4. *Nature*, **227**: 680-685 (1970).
(13) M. M. Bradford: A rapid and sensitive method for the quantitiation microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**: 248-254 (1976).
(14) R. Zrenner , M. Salanoubat, L. Willmitzer and U. Sonnewald: Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants (*Solanum tuberosum* L.). *Plant J.*, **7**: 97-107 (1995).
(15) A. Komatsu, Y. Takanokura, M. Omura and T. Akihama: Cloning and molecular analysis of cDNAs encoding three sucrose phosphate synthase isoforms from a citrus fruit. *Mol. Gen. Genet*., **252**: 346-351 (1996).
(16) H. Hesse, U. Sonnewald and L. Willmitzer: Cloning and expression analysis of sucrose-phosphate synthase from sugar beet (*Beta vulgaris* L.). *Mol. Gen. Genet.*, **247**: 515-520 (1995).
(17) U. Heim, H. Weber and U. Wobus: Cloning and characterization of a full-length cDNA encoding sucrose phosphate synthase from faba bean. *Gene*., **178**: 201-203 (1996).
(18) U. Sonnewald, W. P. Quick, E. MacRae, K. P. Krause and M. Stitt: Purification cloning and expression of spinach leaf sucrose phosphate synthase in *E. coli. Planta*, **189**: 174-181 (1993).
(19) J. Ingram, J. W. Chandler, L. Gallagher, F. Salamini and D. Bartels: Analysis of cDNA clones encoding sucrose-phosphate synthase in relation to sugar inter- conversions associated with dehydration in the resurrection plant *C. plantagineum. Plant Physiol.*, **115**: 113-121 (1997).
(20) J. M. Bruneau, A. C. Worrell, B. Cambou, D. Lando and T. A. Voelker: Sucrose phosphate synthase, a key enzyme for sucrose biosynthesis in plants-Protein purification from corn leaves and immunological detection. *Plant Physiol.*, **96**: 473-478 (1991).
(21) A. T. Chávez-Bárcenas, J. J. Valdez-Alarcón, M. M. Trujillo, L. Chen, B. X. Cázares, W. J. Lucas and L. H. Estrella: Tissue-specific and developmental pattern of expression of the rice sps1 gene. *Plant Physiol*., **124**: 641-654 (2000).
(22) W. L. Chen, D. J. Huang, P. H. Liu, H. L. Wang, J. C. Su and P. D. Lee: Purification and characterization of sucrose phosphate synthase from sweet potato tuberous roots. *Bot. Bull. Acad. Sin*., **42**: 123-129 (2001).
(23) S. J. Carlson, P.S. Chourey, T. Helentjaris and R. Datta: Gene expression studies on developing kernels of maize sucrose synthase (SuSy) mutants show evidence for a third SuSy gene. *Plant Mol. Biol*., **49**: 297-305 (2002).
(24) R. R. Klein, S. J. Crafts-Brandner and M. E. Salvucci: Cloning and develop-mental expression of the sucrose-phosphate-synthase gene from spinach. *Planta*, **190**: 498-510 (1993).