Planta (2001) 214: 250–256
DOI 10.1007/s004250100608

ORIGINAL ARTICLE

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# Sucrose-phosphate phosphatase from *Anabaena* sp. strain PCC 7120: isolation of the protein and gene revealed significant structural differences from the higher-plant enzyme

Received: 30 January 2001 / Accepted: 8 March 2001 / Published online: 11 July 2001


**Abstract** The present study describes the first isolation and characterization of a prokaryotic protein and gene for sucrose-phosphate phosphatase (SPP), the enzyme that catalyzes the terminal step in sucrose synthesis. For gene isolation, a 2,015-bp DNA fragment containing an open reading frame with about 31% amino acid identity to *Synechocystis* SPS was amplified from *Anabaena* sp. PCC 7120 DNA. Surprisingly, expression of the putative gene in *Escherichia coli* demonstrated that it encoded an SPP protein. The expressed protein cross-reacted with antibodies against the native form of *Anabaena* SPP and its biochemical properties were identical to those of the enzyme purified from the cyanobacterial cells. Comparisons of the *Anabaena* SPP with the higher-plant enzyme revealed important differences in the C-terminal region, molecular mass, subunit composition and immunoreactivity. Nevertheless, two conserved motifs, including four invariant aspartate residues similar to those found in members of the phosphohydrolase superfamily, were identified in the *Anabaena* SPP deduced amino acid sequence.

**Keywords** *Anabaena* · Cyanobacterium · Sucrose · Sucrose-phosphate phosphatase · Sucrose-phosphate synthase

**Abbreviations** $M_r$: relative molecular mass · SPP: sucrose-phosphate phosphatase · SPS: sucrose-phosphate synthase

## Introduction

Sucrose metabolism in higher plants has been studied extensively. The most common enzymatic pathway for the synthesis of sucrose involves the production of a phosphorylated intermediate, sucrose-6-phosphate, catalyzed by sucrose-phosphate synthase (SPS, UDP-glucose: D-fructose-6-phosphate glucosyltransferase, EC 2.4.1.14). The hydrolysis of sucrose-6-phosphate to yield free sucrose is catalyzed by sucrose-6-phosphate phosphatase (SPP, sucrose phosphohydrolase EC 3.1.3.24). SPS proteins and genes have been isolated and extensively characterized from various species of higher plants (Huber and Huber 1996). In contrast, the information on SPP is surprisingly limited, and there are only a few reports on the physical, catalytic and regulatory properties of the plant enzyme (Whitaker 1984; Echeverria and Salerno 1994; Lunn et al. 2000). Recently, a biosynthetic pathway for sucrose similar to the pathway in higher plants was identified in two prokaryotic organisms, the cyanobacterium *Anabaena* sp. PCC 7119 and *Synechocystis* sp. PCC 6803 (Porchia and Salerno 1996; Curatti et al. 1998). The present study describes the biochemical and structural analysis of SPP from *Anabaena* sp. strain PCC 7120, a filamentous nitrogen-fixing cyanobacterium. In addition, we report the first isolation of a prokaryotic SPP gene (*spp*A). SPP from *Anabaena* and the enzyme from higher plants differ in polypeptide molecular mass and oligomeric composition. Similarly, the purification, molecular cloning and sequence analysis of SPP from plant sources were recently reported (Lunn et al. 2000).

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## Materials and methods

### Culture conditions

*Anabaena* sp. strain PCC 7120 was grown in BG-11 liquid medium (Rippka et al. 1979) as described by Porchia et al. (1999). Cells of *Escherichia coli* strain DH5α bearing pG750 or pGEM-T easy (+) and strain BL21(λDE):pLysS bearing pR750 or pRSET A (+) were grown in Luria-Bertani medium supplemented with 50 μg/ml carbenicillin.

### Isolation and purification of SPP from *Anabaena* sp. PCC 7120

Packed cells (6–8 g fresh weight) harvested from *Anabaena* cultures at late-exponential-phase were resuspended, disrupted and

centrifuged as described by Porchia and Salerno (1996). The supernatant fraction, referred to as crude extract, was loaded onto a DEAE-Sephacel (Amersham Pharmacia) column and bound proteins were eluted as described by Porchia and Salerno (1996). Fractions with SPP activity, free from SPS and sucrose synthase activities, were pooled, concentrated using an Amicon ultrafiltration cell, and applied to a column of Phenyl-Sepharose 6B Fast-Flow (Amersham Pharmacia) equilibrated with 20 mM Hepes-NaOH (pH 6.5), containing 10 mM $MgCl_2$, 5 mM 2-mercaptoethanol (ME), and 0.85 M $(NH_4)_2SO_4$. Proteins were eluted with a linear $(NH_4)_2SO_4$ gradient from 0.85 to 0 M in equilibration buffer. Fractions containing SPP activity were applied to a Sephadex G-75 (Amersham Pharmacia) column equilibrated with 50 mM Hepes-NaOH (pH 7) containing 10 mM $MgCl_2$, 5 mM ME, and 10% (v/v) glycerol. Additionally, this enzyme fraction was purified further by preparative non-denaturing PAGE and the protein gel band with SPP activity was used to raise polyclonal antibodies in rabbits (Salerno et al. 1998).

### Effect of bivalent ions

To aliquots of the Sephadex G-75 pool of SPP, EDTA was added to a final concentration of 100 mM. Enzyme fractions were agitated in a bath at 0 °C for 30 min and exhaustively dialyzed against 10 mM Hepes-NaOH (pH 7) before assay at different $Mg^{2+}$ or $Mn^{2+}$ concentrations as described by Porchia et al. (1999).

### Polyacrylamide gel electrophoresis

Proteins were separated by SDS-PAGE on 12 or 15% polyacrylamide gels (Laemmli 1970) and visualized with Coomassie blue or silver staining (Bio-Rad kit). To ascertain the SPP polypeptide position, a gel was made so that half was stained and the other half was used for determining SPP activity in-gel after removal of SDS and renaturation (Kameshita and Fujisawa 1989; Porchia and Salerno 1996).

### Western immunoblots and immunotitration of SPP activity

Proteins were separated by 15% SDS-PAGE and electroblotted onto a nitrocellulose membrane (HyBond C; Amersham) as described by Renart and Sandoval (1984). The membranes were then probed with antibodies raised in rabbits against native *Anabaena* SPP (anti-*Anabaena* SPP) according to Salerno et al. (1998).

The concentration dependence of SPP activity inhibition by anti-*Anabaena* SPP was determined by adding different volumes of antibodies to aliquots of purified *Anabaena* SPP or the recombinant ($His_6$::SPP) protein as previously described (Salerno et al. 1991). Parallel incubations with preimmune serum or rice leaf SPP (Echeverría and Salerno 1994) were carried out as a control. After incubation, SPP activity was determined in the supernatant fluids.

### Enzyme assays

SPP activity was determined by measuring Pi that was released following the procedure of Echeverría and Salerno (1993). Crude extracts were desalted through a Sephadex G-50 column before the enzyme activity assay. SPS and sucrose synthase activities were measured as previously described (Porchia and Salerno 1996; Porchia et al. 1999). Proteins were quantified according to Bradford (1976) or by absorbance at 280 nm.

### Amplification, cloning and identification of the *spp* gene

High-molecular-mass genomic DNA was isolated from *Anabaena* sp. strain PCC 7120 cells as described by Cai and Wolk (1990) and plasmids were isolated and modified according to standard protocols (Sambrook et al. 1989). A 2,015-bp DNA fragment containing an open reading frame of 750 bp (750 *orf*) flanked by 850 bp upstream and 415 bp downstream was amplified using polymerase chain reaction (PCR) methodology from DNA of *Anabaena* sp. strain PCC 7120 (http//www.kazusa.or.jp/cyano/) with the following forward (5′-GCCGTCGGGAATCTGAAATACAGCGTATA-3′) and reverse (5′-CGATGGATGGTTAGGAGTGAGTTTT-3′) primers. The PCR conditions were as previously described (Curatti et al. 2000). PCR products were analyzed by electrophoresis in 1% (w/v) agarose gels in Tris-acetate buffer and ethidium bromide staining (Sambrook et al. 1989), and ligated to vector pGEM-T Easy (Promega, Madison, Wis., USA); the resulting pG750 was used for transforming *E. coli* DH5α. Overnight cultures of clones with SPP activity were harvested by centrifugation. Cells were resuspended in five times their packed volume of extraction buffer (Porchia and Salerno 1996) and broken by passage through a French press at 700 psi. Cell debris was removed by centrifugation and the supernatant fraction was chromatographed on a DEAE-Sephacel column (Pharmacia) as described by Porchia and Salerno (1996).

### Purification of $His_6$-tagged SPP ($His_6$::SPP) expressed in *E. coli*

For overexpression of SPP, the 750 *orf* was amplified under PCR conditions similar to those described above with the following forward (5′-CGGGATCCATGAAGCCATTTCTTTTTGTCA-3′) and reverse (5′-CGGAATTCTCATTCGAGGAAAC-CAAAGAT-3′) primers. The amplification product was ligated between the *Bam*HI and *Eco*RI sites of the expression vector pRSET-A (Invitrogen Corporation, San Diego, Calif., USA). The resulting construct (pR750) was introduced into *E. coli* strain BL21(λDE3):pLysS (Novargen/AGS, Heidelberg, Germany). Protein expression was induced by addition of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). After incubation at 28 °C for 18 h, cells were harvested by centrifugation. The pellet was resuspended in lysis buffer [20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF)], and subjected to three freeze-thaw cycles. The crude lysate was centrifuged at 27,000 *g* for 30 min at 4 °C and the resulting supernatant fluid was used as enzyme source. One-step affinity purification of the fusion protein tagged ($His_6$::SPP) employed the TALON immobilized $Co^{2+}$ resin (Clontech Lab. Inc., Palo Alto, Calif,. USA). The bound protein was eluted under native conditions with imidazole lysis buffer as described by the manufacturer. The purified recombinant enzyme was concentrated and used for further characterization studies.

### Sequence analysis

For initial DNA comparison analysis, sequences were compiled and analyzed using the BLASTp protocol (Altschul et al. 1990) and the Clustal W method running the Megalign program of the DNAStar package with the PAM 250 residue weight table. Comparisons were performed with data obtained from GenBank and EMBL databases. The DNA alignment shown in the present paper was generated with the ProDom program, using the BLOSUM 62 matrix.

## Results

### Purification of SPP from *Anabaena* and molecular mass determination

SPP was highly purified from *Anabaena* sp. PCC 7120 cells according to the purification protocol presented in Table 1. A final purification of about 3,260-fold was achieved after three chromatographic steps. The Sephadex G-75 concentrated fraction showed a major polypeptide band of $27 \pm 1$ kDa when subjected to SDS-PAGE (Fig. 1A, lane 4). The identity of the SPP

**Table 1** Purification of SPP from *Anabaena* sp. PCC 7120[a]

| Step | Protein (mg) | Total activity (nkat) | Specific activity (nkat (mg protein)$^{-1}$) | Recovery (%) | Purification (-fold) |
|---|---|---|---|---|---|
| Crude Extract | 75 | 6.22 | 0.08 | 100 | 1 |
| DEAE-Sephacel | 8.5 | 5.35 | 0.63 | 86 | 8 |
| Phenyl Sepharose | 0.4 | 3.17 | 7.90 | 51 | 99 |
| Sephadex G-75 | 0.009 | 2.35 | 261 | 38 | 3.260 |

[a]See Fig. 1

polypeptide was confirmed by measuring enzyme activity in a parallel lane of the gel after renaturation (Fig. 1B).

SPP exhibited maximum activity at pH 6.5 and its native $M_r$ was estimated to be $26 \pm 2$ kDa by gel filtration chromatography (data not shown). A similar biochemical characterization was obtained with preparations from *Anabaena* sp. strain PCC 7119 (data not shown).

## Substrate specificity and effect of cations and inhibitors

Preparations of *Anabaena* SPP showed no activity with 1–5 mM glucose-1-phosphate, fructose-6-phosphate, fructose-1,6-bisphosphate, glucose-1,6-bisphosphate and trehalose-6-phosphate as compared to sucrose-6-phosphate. The apparent $K_m$ for sucrose-6-phosphate calculated from a Wolf plot is $0.35 \pm 0.10$ mM. Either $Mg^{2+}$ or $Mn^{2+}$ increased enzyme activity and the calculated activation constants in Wolf plots were $0.5 \pm 0.1$ mM and $5 \pm 1$ mM, respectively (data not shown). The effects of common phosphatase inhibitors (molybdate, vanadate and fluoride) were tested on SPP activity. At concentrations less than 35 mM, vanadate had a stronger inhibitory effect than the two other inhibitors (Fig. 2). While 2.5 mM vanadate inhibited SPP activity about 50%, molybdate and fluoride only inhibited activity by about 12 and 5%, respectively. Sucrose was a weak inhibitor (Fig. 2) and a competitive inhibition was found [$K_{i(sucrose)}$ of $80 \pm 20$ mM (data not shown)].

## Isolation and identification of the *Anabaena* SPP gene

In our current investigation of sucrose biosynthesis in prokaryotic organisms, a BLAST sequence similarity search (Alschul et al. 1990) with the sequence of the *Anabaena* sp. PCC 7120 genome (Kazusa DNA Research Institute in the Cyanobase) revealed the existence of *orf*s with homology to *sps*A, the gene coding SPS from *Synechocystis* sp. PCC 6803 (Curatti et al. 1998). One of them, named 750 *orf*, was of interest because: (i) protein extracts from *E. coli* harboring pG750 showed a very low SPS activity; (ii) its deduced amino acid sequence shared 31% identity with the carboxy-terminal domain of *Synechocystis sps*A; and (iii) the deduced amino acid sequence corresponded to a polypeptide of 28,034 Da, which was considerably smaller than *Anabaena* SPSs (Porchia and Salerno 1996). These findings, together with the *Anabaena* SPP $M_r$ of $\approx$27 kDa (see above) and the reported phosphatase domain in the carboxy-terminal region of trehalose-phosphate-synthase (Goddijn and van Dun 1999), led us to examine the possibility that the 750 *orf* might encode a protein with SPP activity. This presumption was confirmed by showing that extracts of *E. coli* harboring pG750

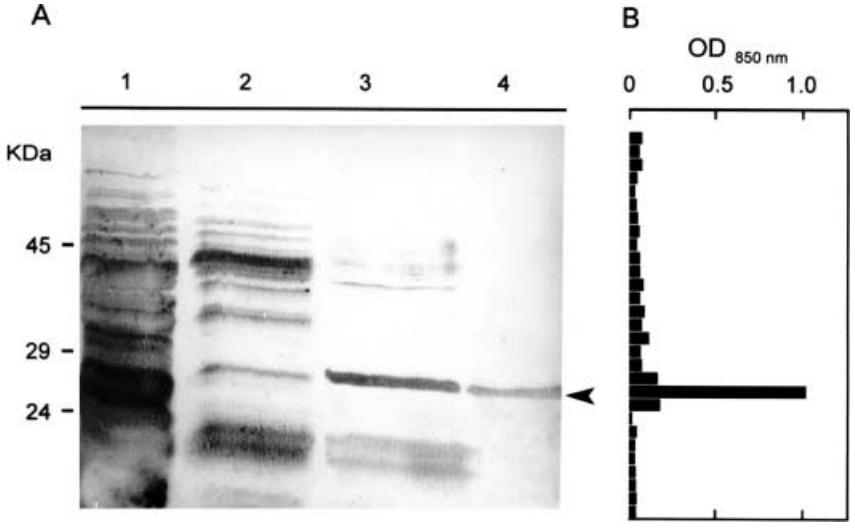


**Fig. 1A, B** SDS-PAGE analysis of SPP from *Anabaena* sp. PCC 7120 at various stages of purification. **A** Silver staining. *Lane 1* Crude extract, *lane 2* DEAE-Sephacel, *lane 3* Phenyl Sepharose, *lane 4* Sephadex G-75. **B** SPP activity determined after renaturing a parallel lane loaded with an aliquot of the Sephadex G-75 fraction. *Arrowhead*, position of the SPP polypeptide

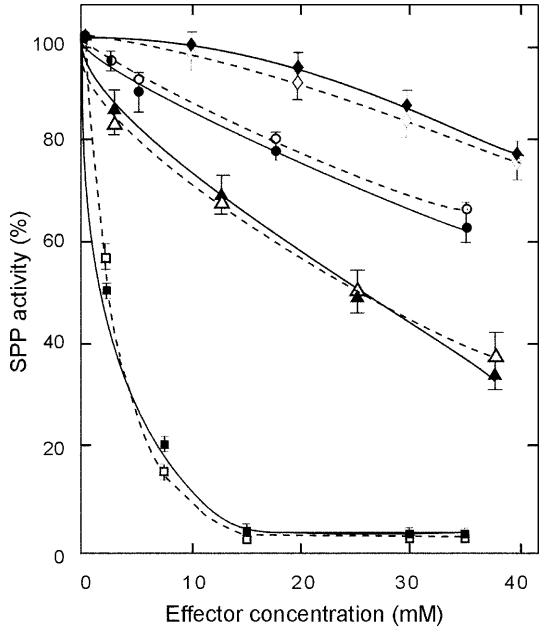


**Fig. 2** Effect of common phosphatase inhibitors and sucrose on *Anabaena* SPP activity. ■, □ Vanadate; ▲, Δ molybdate; ●, ○ fluoride; ◆, ◇ sucrose. *Closed symbols*, *Anabaena* SPP; *open symbols,* recombinant SPP. Values are means ± SD of three replicates

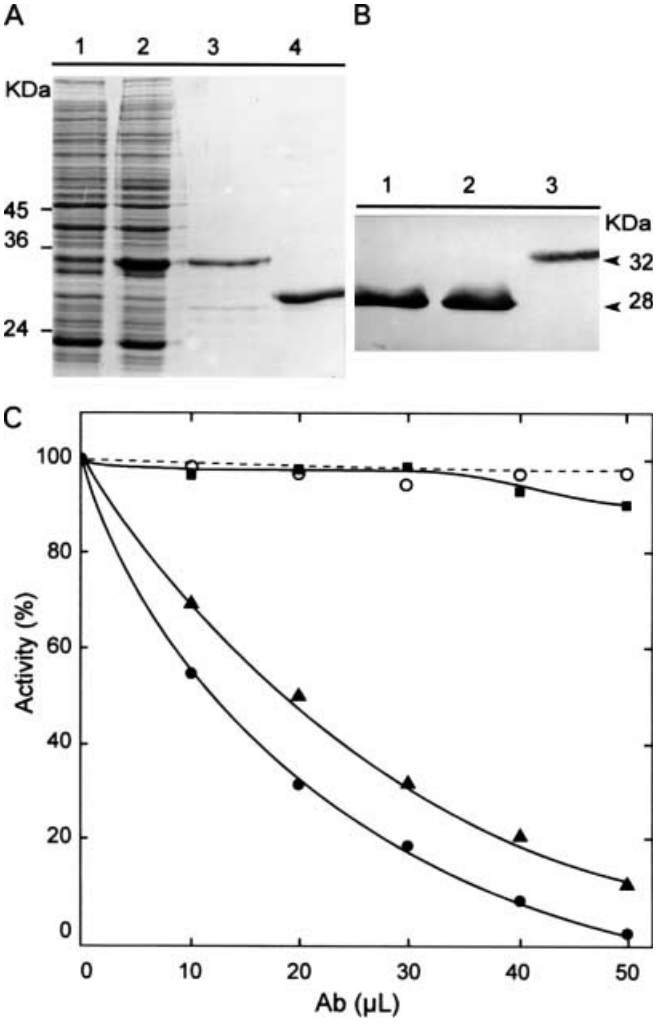


**Fig. 3A–C** Expression of *Anabaena spp*A in *E. coli* and immunoanalysis of the recombinant $His_6$::SPP. **A** Coomassie blue staining after SDS-PAGE on a 12% gel of the recombinant protein at different purification stages. *Lane 1* Crude extract from non-induced *E. coli* cells (20 μg), *lane 2* crude extract from IPTG-induced cultures (20 μg), *lane 3* $His_6$::SPP purified by metal-ion affinity chromatography (1.5 μg), *lane 4* Sephadex G-75 *Anabaena* SPP fraction (3 μg). **B** Western blot using anti-*Anabaena* SPP. *Lane 1* Sephadex G-75 *Anabaena* SPP fraction, *lane 2* DEAE-Sephacel recombinant protein expressed in *E. coli* harboring pG750, *lane 3* purified $His_6$::SPP. **C** Concentration dependence of SPP activity inhibition by anti-*Anabaena* SPP, following addition of the indicated volumes of anti-*Anabaena* SPP to aliquots of Sephadex G-75 *Anabaena* SPP (●–●), $His_6$::SPP (▲–▲), and rice leaf SPP (■–■). *Dotted line*, control incubation of *Anabaena* SPP with pre-immune serum (○–○)

exhibited mainly SPP activity [about 13 nkat (mg protein)$^{-1}$ as specific phosphohydrolase activity in comparison with approx. 0.1 nkat (mg protein)$^{-1}$ as SPS activity], whereas *E. coli* bearing pGEM-T Easy had neither SPP nor SPS activity. Moreover, partial purification of the recombinant enzyme through a DEAE-Sephacel column showed that SPP activity eluted at an NaCl concentration (0.20 M) similar to that of the authentic *Anabaena* SPP protein (data not shown).

Further characterization of the 750 *orf* was achieved with the purified recombinant protein obtained using the $His_6$-tagged system and the plasmid pR750. The $His_6$::SPP protein eluted from the metal-ion affinity chromatography column (Fig. 3A, lane 3) was used for biochemical studies. Similar to authentic *Anabaena* SPP, $His_6$::SPP hydrolyzed sucrose-6-phosphate, exhibited a pH optimum of 6.5, an apparent $K_m$ value of $0.50 \pm 0.15$ mM, activation by bivalent cations (data not shown), and inhibition by sucrose (competitive inhibitor, $K_i$ $87 \pm 15$ mM) and common phosphatase inhibitors (Fig. 2). Also, antibodies raised against the *Anabaena* SPP recognized a 28-kDa polypeptide in the Sephadex G-75 SPP fraction following separation of the authentic *Anabaena* enzyme, and polypeptides of 28 kDa and 32 kDa corresponding to the recombinant proteins expressed from pG750 and pR750 ($His_6$::SPP protein), respectively (Fig. 3B, lanes 1–3). Moreover, the antibodies removed the $His_6$::SPP protein activity from solution in a concentration-dependent way, similar to the *Anabaena* SPP activity. In contrast, when the antibodies were added to a rice leaf SPP preparation (Salerno et al. 1998), the total enzyme activity remained in the supernatant fraction (Fig. 3C). Taken together, these results lead us to conclude that the 750 *orf* corresponds to an SPP gene (*spp*A).

## Sequence analysis of *Anabaena* SPP protein

The *Anabaena spp*A gene encodes a 249-amino-acid polypeptide with a predicted $M_r$ of 28,034 Da. This value

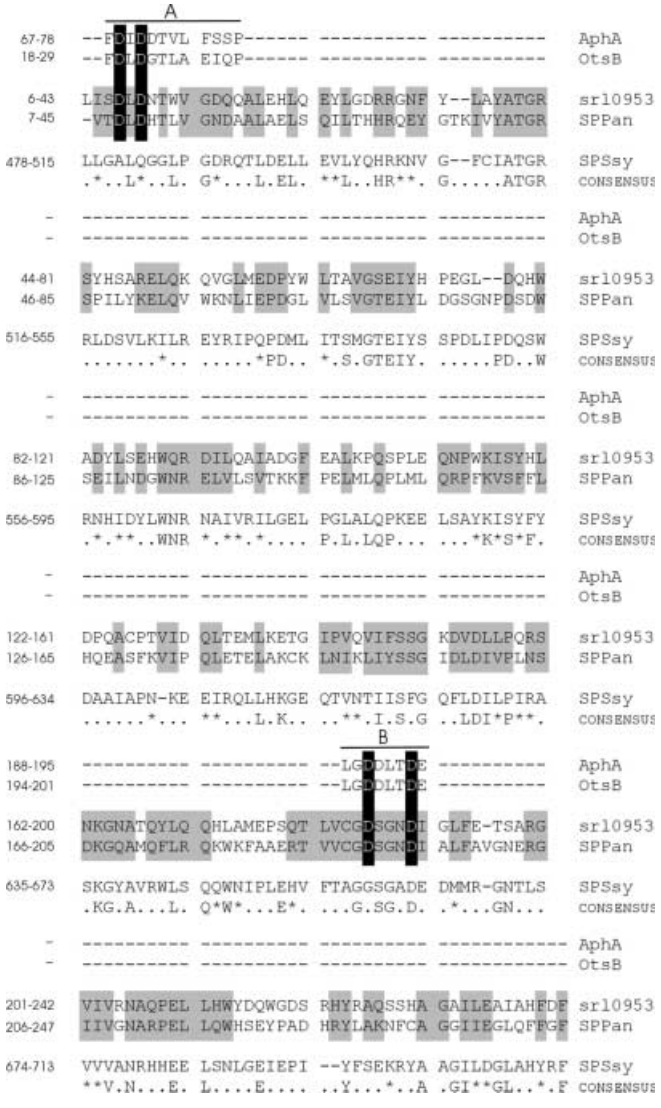


**Fig. 4** Amino acid sequence comparison of cyanobacterial SPPs and SPS, and presence of sequence motifs (*A* and *B*) of phosphatases. Identical residues and conservative amino acid substitutions between the deduced sequences from *Anabaena* sp. PCC 7120 SPP (*SPPan*; AJ302073) and *Synechocystis* sp. PCC 6803, a putative SPP (*srl0953*) are in *grey shading*. Two sequence motifs (*A* and *B*) shared by phosphatases are included by alignment of the deduced amino acid sequences corresponding to OtsB (trehalose-phosphate phosphatase, AAC 74967) and AphA (deadenosine tetraphosphatase, AAC 77025). The presence of four invariant aspartate residues is indicated by *black shading*. The consensus between *Synechocystis* SPS (*SPSsy*, residues 478–713; BAA 10782) and *Anabaena* SPP is represented by *capital letters* for identical amino acid residues, and *asterisks* for conservative substitutions. The alignment was generated with the ProDom program, using the BLOSUM 62 matrix

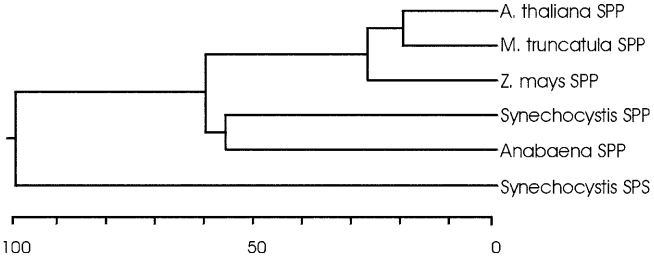


**Fig. 5** Phylogenetic tree of SPPs. Predicted SPP sequences were obtained from the GenBank database. The tree was generated with the program Megalign of the DNAStar package, using the Clustal W method with the PAM 250 residue weight table. The deduced sequences are from *Anabaena* sp. PCC 7120 SPP (AJ302073), the *Synechocystis* sp. PCC 6803 putative SPP (srl0953, AF 300455), *Arabidopsis thaliana* SPP (AF 283565), *Zea mays* SPP (AF 283564), and *Medicago truncatula* SPP (AF 283566)

is similar to the $M_r$ of the native protein determined by gel-filtration chromatography (data not shown) and to the $M_r$ of the polypeptide calculated after SDS-PAGE electrophoresis (Fig. 1A, lane 4), indicating that *Anabaena* SPP is a monomeric protein. A comparison of the deduced amino-acid sequence of *Anabaena spp*A with those of *Synechocystis spsA* (Curatti et al. 1998) and the *orf* detected in the *Synechocystis* genome (Kaneko et al. 1996) as a putative-SPP gene (slr0953; Lunn et al. 2000) is presented in Fig. 4. A dendogram generated by multiple alignments, using the Clustal W method, shows that the cyanobacterial sequences clearly diverge from the plant SPPs (Fig. 5) reported recently (Lunn et al. 2000).

## Discussion

This study completes the initial characterization of the enzymes of the sucrose biosynthetic pathway in prokaryotic organisms (Salerno et al. 1995; Porchia and Salerno 1996; Curatti et al. 1998, 2000; Porchia et al. 1999). To our knowledge, the present work represents the first biochemical characterization of a prokaryotic SPP, the enzyme catalyzing the terminal step in sucrose synthesis, and the identification of its gene (*spp*A) from *Anabaena* sp. strain PCC 7120.

*Anabaena* SPP was highly purified (about 3,260-fold) from cells growing in the presence of combined nitrogen (Table 1; Fig. 1), strengthening the view that sucrose synthesis can occur independently of nitrogen fixation. The cyanobacterial enzyme, similar to the enzyme from higher plants, is highly specific, unable to cleave phosphate esters other than sucrose-6-phosphate at a significant rate. The apparent $K_m$ value of *Anabaena* SPP was 350 μM, somewhat higher than estimates of the substrate affinity of plant SPPs (35–250 μM) (Hawker and Hatch 1966; Whitaker 1984; Krause and Stitt 1992; Echeverría and Salerno 1994). The biochemical characterization of *Anabaena* SPP revealed similar properties to plant SPPs (e.g., a pH optimum of 6.5, activation by $Mg^{2+}$ and $Mn^{2+}$, inhibition by vanadate, molybdate and fluoride; Fig. 2). Additionally, *Anabaena* SPP was modestly sensitive to inhibition by high concentrations of product ($K_{i(sucrose)} = 80$ mM), in agreement with most plant SPP reports (Hawker and Hatch 1966; Whitaker 1984; Krause and Stitt 1992; Echeverria and Salerno 1993, 1994; Lunn et al. 2000). The dephosphorylation of

sucrose-6-phosphate per se does not appear to be the regulatory step in the sucrose biosynthesis pathway in *Anabaena* since inhibition of SPP by sucrose was low and required sucrose concentrations much higher than those that occur physiologically (Porchia and Salerno 1996). A possible point of sucrose synthesis regulation in cyanobacteria may be the interaction between SPS and SPP, allowing the channeling of the transglucosylation product of SPS (sucrose-6-phosphate) towards SPP, as has been proposed in plants (Echeverria et al. 1997).

The difference in native $M_r$ between *Anabaena* and plant SPPs is notable. Unlike the enzyme in plants, where the native SPP form was reported as a homodimer (Whitaker 1984; Echeverría and Salerno 1994; Lunn et al. 2000), the native *Anabaena* SPP protein is composed of a single polypeptide chain with an $M_r$ of about 28,000 Da determined by gel filtration and SDS-PAGE (Figs. 1, 3). The predicted $M_r$ of the SPP polypeptide, was 28,034 Da, a value that is approximately half that of the eukaryotic SPP subunit (Lunn et al. 2000). An important overall structural difference among cyanobacterial sucrose biosynthetic enzymes is that they have a monomeric composition (Porchia and Salerno 1996; this paper), whereas SPS and SPP from higher plants are multimers composed of larger polypeptides (Whitaker 1984; Salvucci et al. 1990; Echeverría and Salerno 1994; Huber and Huber 1996; Salerno et al. 1998; Lunn et al. 2000). These differences may explain their distinct catalytic capacities. Thus, the specific activity of *Anabaena* SPS (Porchia and Salerno 1996) is about 1–20% of those of the purified plant SPSs (Salvucci et al. 1990; Sinha et al. 1997) and the specific activity of *Anabaena* SPP (this paper) is 1–3% of that reported for the plant rice enzyme (Lunn et al. 2000). In addition, the apparent $K_{a(Mg2+)}$ for *Anabaena* SPP (0.5 mM) is 50-fold higher than that of rice SPP (Lunn et al. 2000).

A 750-bp *orf* present in the *Anabaena* sp. strain PCC 7120 genome was identified as the coding region of the SPP gene (*spp*A) as its expression in *E. coli* produced a fully active SPP (249-amino-acid polypeptide) whose kinetic and molecular characteristics were similar to those of SPP purified from *Anabaena* cells (Figs. 2, 3). The deduced amino-acid sequence of *spp*A showed 31% identity with the carboxy-terminal domain of *Synechocystis sps*A (residues 478–713; Fig. 4). Additionally, *spp*A expressed in *E. coli* produced a protein with SPS activity, although of very low activity in comparison with its specific phosphohydrolase activity. The proposed SPS-substrate domains (Salvucci et al. 1995) are located in the amino-terminal region of plant SPSs (spinach residues 197–204 and 227–240) and *Synechocystis* SPS (residues 30–37 and 60–73) (Curatti et al. 1998); therefore, SPS activity measured in the recombinant SPP may indicate that the carboxy-terminal region of SPS perhaps plays a role in the catalytic activity of SPS.

Recently, conserved sequence motifs have been identified among bacterial, eukaryotic and archaeal phosphatases that define a new phosphohydrolase superfamily (Thaller et al. 1998). Those motifs, clustered within two domains (A and B) separated by a variable spacer region, according to the general pattern D-X-D-X-[TV]-X(109,198)-G-D-X(3)-D, were also found in *Anabaena* SPP (Fig. 4). Consequently, SPP should be considered a member of the superfamily of phosphohydrolases indicated as "DDDD" after the presence of the four invariant aspartate residues. Additionally, *Anabaena* SPP also shows the three motifs typical of members of the HAD superfamily of proteins (Aravind et al. 1998) as was reported for the maize enzyme (Lunn et al. 2000).

Taking our results together with the sequence of the recently reported plant SPPs (Lunn et al. 2000), we generated a dendogram showing that the cyanobacterial enzymes clearly diverge from plant SPPs (Fig. 5). The overall amino-acid identity between plant SPP and the *Synechocystis* putative SPP is lower (about 30%) than that between plant and *Anabaena* SPP (about 38%). On the other hand, while the identity between plant SPPs is about 50–65% (Lunn et al. 2000), the cyanobacterial SPPs are only approximately 36% identical (Figs. 4, 5). Collectively, these results are in agreement with the phylogenetic relationship between cyanobacteria and plant chloroplasts (Giovannoni et al. 1988).

*In conclusion*, the results presented herein confirm that SPS and SPP are also responsible for sucrose biosynthesis in cyanobacteria. In addition, these findings will certainly contribute to a better understanding of the evolution of the "modern" sucrose-metabolizing enzymes present in higher photosynthetic organisms. The phylogenetic relationships between SPPs and SPSs from both plants and cyanobacteria led us to hypothesize that modern cyanobacterial and plant SPPs could have arisen from a common ancestral gene that may be related to an ancestral SPS gene.

**Acknowledgements** The authors thank L. Curatti and D. Fiol for helpful discussions, L. Giarrocco and C. Fernández, for technical assistance. This research was supported by grants to G.L.S. from the Consejo Nacional de Investigaciones Cientificas y Tecnológicas, ANPCyT, FIBA, and Universidad Nacional de Mar del Plata. G.L.S. is a Career Investigator (CONICET). This work is part of the Ph.D. thesis of A.C., Universidad Nacional de Mar del Plata, Argentina.

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