The Activities of SPS and SPP Enzymes for Sucrose Synthesis in Storage Tissues of Sugarcane

Charuwan Bangwaek[1] Takayoshi Terauchi[2] Maribel Quintana[2]

ABSTRACT

Sucrose phosphate Synthase (SPS) and Sucrose phosphate phosphatase (SPP) has been reported in many plants associated with sucrose synthesis. The activities of these enzymes are also significant to indicate the sugarcane ability to accumulate surcose in storage tissue. This study showed that SPS and SPP are synergistic enzyme to synthesize sucrose in sugarcane. They displayed much higher activities when they work together than each individual. SPS and SPP enzymes effected sucrose content. It showed sucrose accumulation increased with higher amounts of SPS and SPP enzymes in all sugarcane varieties tested (NC0310 and F172). However, different types of sugarcane varieties between commercial and wild canes have different efficiency of these enzymes.

Keywords: Enzyme activity, sugar accumulation, sugarcane cultivars, phosphate sythase, sucrose phosphate phosphatase, sucrose synthase

1. Prachinburi Rice Research Center

2.Okinawa Subtropical Station, Japan International Research Center for Agricultural Science, Ishigaki 907200, Japan

Introduction

Sucrose is the majority of sugar stored in the vacuoles of stem in sugarcane. Sucrose can account for up to 50 % of the total dry weight in older internodes, reaching a concentration of 500 mM (Glasziou and Gaylor, 1972)

Sucrose translocates from the leaves to various sink tissues. The part of the translocated sucrose was hydrolysed and resynthesised in the storage sink during sucrose accumulation (Hatch and Glaziou, 1964). Stitt *et al.* (1987) reported that sucrose synthesis occurs via the sequential action of sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP). Echeverria and Salerno (1993) found that SPP is the second enzyme in the 2 steps of sucrose synthesis. SPS pathway leading to *de novo* sythesis of sucrose in plant cells. SPS is known as an enzyme which synthesize sucrose phosphate (Suc-P) from Fructose-6-phosphate (F-6-P), UDP-Glucose (UDPG) with G-6-P as activator. SPP leads to the formation of sucrose from sucrose phosphate.

SPS and SPP are known as sucrose synthetic enzymes. It was reported in many papers that SPS is found in photosynthetic as maize leaves (Worrell *et al.*, 1991), lettuce leaves (Echeverria and Salerno, 1993), spinach leaves (Sulvucci *et al.*, 1990), potato (Tobias *et al.*, 1999) etc. There are few papers that reported about SPS and SPP enzymes in non-photosynthetic tissues. Moore (1995) reviewed about sugar accumulation in sugarcane. Hubbard *et al.* (1989) reported that muskmelon accumulate soluble sugars during ripening contain SPS and activity of this enzyme increase during the period of sugar accumulation. Addition, Hubbard *et al.* (1989) reported that in fruits such as peach, strawberry, kiwi contained significant activities of sucrose phosphate synthase activity.

There are few reports about SPP. Some researchers hypothesized that SPS forms a complex with SPP for synthesizing sucrose and transferring to vacuoles where the sucrose accumulates.

This study purpose to understand the roles of activities of SPS and SPP enzymes related to sucrose synthesis in storage tissues of sugarcane.

Materials and Methods

1. Plant materials

The fifteenth internodes from top full-expanded leaf of 2 sugarcane commercial varieties; NC0310 andF172. Ten plants of each variety were harvested and applied for the test in January 1999. Three wild type varieties, *S. Spontaneum* vars.,Truk, SES205 and Timor wild, were also sampled of SPS assay. These samples were sliced to less than 1 mm. after rind removal. Part of each sample was squeezed to obtain juice for sugar analysis, the remainder was stored in a -80°C freezer after freezing by liquid nitrogen.

2. Sugar analysis

Juice samples were dilutied to 10% with 80% ethanol. Sucrose, glucose, and fructose concentrations were measured by HPLC (High Pressure Liquid Column) with a separator column (Shodex CLC-NH_2P) and a detector (Refraction Index Detector) with 65% acetronitrile as carrier in flowing rate 1 ml min^{-1}. Brix was also measured using a hand reflectrometer.

3. Enzyme preparation

The procedures of enzyme extraction were

the same for SPS and SPP in different buffers. Two grams of frozen sugarcane were ground and homogenized with 4 ml of extraction buffer. The homogenate was filtered using cotton wool and centrifuged at 15000 g for 10 minutes. The resulting supermatant was desalted through a sephadex G-25 column with desalting buffer. The desalted solution was used as the enzyme source. All the above operations were conducted at 0-4°C.

4. Enzyme activity

The activities of enzymes, SPS and SPP, were detected for 6 replications.

(a) SPS

A buffer of 50 mM MOPS-NaOH pH 7.5, 10 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 0.1% (W/V) Triton X-100 was used for SPS extraction

The 70 ul of desalted extract was mixed into 3 ul of reaction mixture that contains 10 mM F-6-P, 40 mM G-6-P, 10 mM UDPG and 10 mM KH_2PO_4 and incubated at 37°C for 1 hour. The reaction was halted by cold temperature and addition of 30% KOH. The samples were boiled for 10 minutes. The sucrose phosphate formation was measured by using anthrone sulfuric acid methods detecting light absorbance at 620 nm (van Hande, 1968).

(b) SPP

A buffer of 100 mM HEPES (pH 7.5), 20 mM $MgCl_2$, 2mMEDTA, 5 mM PMSF (Phenylmethyl sulphonyl fluoride), and 2% ethyleneglycol was used for SPP extraction (Echeverria and Salerno, 1993).

SPP activity was examined using a measurement of inorganic phosphate (Pi), which was produced in the enzyme reaction. 40 ul of desalted extract was mixed with 10 ul of reaction mixture contained 2 mM sucrose-P and 50 mM HEPES (pH 7.5) and incubated at 30°C for 60 minutes. After incubation, 100 ul of 35 mM EDTA was added to stop the reaction. The samples were boiled for 10 minutes. Pi was measured following the method of Chifflet *at al.* (1988).

(c) the enzyme combination test

Reaction mixture for SPS activity (30 ul) described above was added with 70 ul of enzyme extracts for SPS, incubated at 37°C. 1 hour. And then 100 ul of SPP extract was added and incubated at 30°C for 60 minutes. The reaction was stopped by cooling in ice and 10 ul of 35 mM EDTA. Then samples were boiled for 10 minutes. Pi concentration of samples was determined. Boiled SPS and SPP extracts were used for control.

Results

1. SPS enzyme activity in sugarcane

There was low relationship between sucrose synthesis and SPS activity. The small negative correlation between SPS and sucrose concentration ($r < 0.23$) was observed in both varieties (*S. officinarum*) (Table 4).

There was inter-variety variation on sucrose concentration on NC0310 and F172. SPS activities were different between varieties even though they had similar sucrose content.

Varietal differences of the activities of SPS are detected. The activities of SPS enzymes were also depended upon variety, NC0310 had higher activity F172 (Table 1).

2. SPP enzyme activity in sugarcane

The enzyme activities were indicated in Table 1 in three sucrose concentration level. Table 1 showed sucrose content in sugarcane was higher when SPP activity was positive correlation between sucrose concentration and SPP activity 0.79** (Table 4). This showed that the activity of SPP had higher relationship to sucrose concentration than that of SPS in this season. Consequently, SPP was the dominant enzyme, effected on sucrose content and less sensitive to environmental factors.

The activity of SPP enzyme was depended upon the varieties, as well as SPS. SPP activity in NC0310 was higher than var. F172.

3. The differences of activities of SPS and SPP in commercial and wild canes.

SPS activities were different among the species of sugarcane. The activity of SPS in *S. spontaneum* vars., Truk and SES205 were lower than the detecting level (Table 2). Among wild varieties, had the highest SPS activity. It showed that parallel relationship between SPS activity and sucrose concentrations especially in wild canes. Remarkably the activity of SPS enzyme were highly related with sucrose content in wild canes than commercial variety.

The activity of SPP enzyme in *S. spontaneum* was significantly lower than commercial variety, F172. There were no differences among varieties of *S. spontaneum*.

4. The co-activities between SPS and SPP enzymes

The activities of SPS and SPP appeared to be synergistic to synthesize sucrose (Table 3). The concentration of sucrose was increased to 9.85 mM along with the increment of the 2 enzymes SPS and SPP, compare to each enzyme alone (SPS = 2.35 mM, SPP = 0.17 mM).

Discussions

This result agreed that both SPS and SPP enzymes were required for sucrose synthesis in sugarcane but depended upon the types of sugarcane.

SPS had lower relationship with sucrose content in commercial canes than wild canes. The reason was explained that the sensitivities of SPS enzyme activity of commercial varieties were increase when effect on external factors, the sucrose content result higher than those in wild canes. Otherwise, SPS enzyme in wild canes were stronger controlled by genes than commercial verities. Stitt *et al.* (1988) reported that the extractable activity of SPS increases in parallel with the rate of photosythesis in barley leaves as the light intensity was increase. Many factors effect the activities of SPS were such as light (Pollock and Hously, 1985), osmotic stress (Toroser and Huber, 1997) etc.

Sucrose content were shown stronger responded to SPP enzyme activity than SPS enzyme in all canes tested. Appearance, sucrose content had higher relationship with the activities of SPP than SPS enzymes. The sucrose concentration was increase when the SPP enzyme activities of commercial cane varieties were increase.

In wild canes, the activities of both SPS and SPP enzyme affected stronger positive sucrose content than commercial varieties.

SPS and SPP were related to sucrose synthesis and work as co-active enzymes. Since the fluctuation of sucrose content were not definitely different even SPS activities in some varieties

because SPP enzyme was compromising

The activities of these enzymes were different between commercial varieties. SPS were more sensitive to adverse environment than SPP. However, genes controlled them. The gene expression of SPS and SPP activities were strongly high in the varieties and types of sugarcane and less sensitive to external factors, should be recognized. Further studies should be emphasized in genes controlling the activities of SPS and SPP enzymes for increasing sucrose content in sugar cane.

Table 1. Enzyme activities of commercial sugarcane with various sucrose content.

Verieties	Sucrose content nM	SPS (Suc-P,mM g^{-1} F.W.min^{-1})	SPS (Pi, uM g^{-1} F.W.min^{-1})
NC0310	196.49	2.23 + 0.10	62.78 + 1.37
	376.90	2.37 + 0.30	74.89 + 2.19
	431.29	1.13 + 0.06	93.20 + 1.92
Average	334.89	1.91	76.96
F172	288.60	0.59 + 0.10	61.77 + 1.87
	300.29	1.42 + 0.32	-
	402.92	0.97 + 0.10	73.11 + 1.16
Average	330.60	0.99	67.44

- No data

The data was shown in mean + standard error from 6 replications.

Table 2. SPS activities of sugarcane from S. spontaneum compared to commercial variety F172

Verieties	Sucrose content nM	SPS (Suc-P,mM g^{-1} F.W.min^{-1})	SPP (Pi, uM g^{-1} F.W.min^{-1})
F172	475.1	9.07 + 0.34	34.29 + 0.34
S. spontaneum Var. Truk	135.7	*	12.34 + 0.49
S. spontaneum Var. SES205	157.9	*	10.36 + 0.24
S. spontaneum Var. Timor wild	397.1	1.93 + 0.04	14.33 + 0.31

* Enzyme activity was not detected

The data was shown in mean + standard error from 6 replications.

Table 3. The sucrose synthesis from various types of enzymes SPS and SPP of sugarcane var. F172.

Treatment	SPS : SPP ul : ul	Sucrose mM(70 ul SPS + 100 ul SPP)
SPS + SPP	70 : 100	9.85 + 4.80
SPS (boiled) + SPP	70 : 100	0.17 + 0.17
SPS + SPP (boiled)	70 : 100	2.53 + 0.97
SPS (boiled) + SPP (bioled)	70 : 100	0.77 + 0.06
SPS	70 : 0	0.07 + 0.06
SPP	0 : 100	0.48 + 1.03
SPS (boiled)	70 : 0	ND
SPP (boiled)	0 : 100	2.19 + 0.86

ND – not detected

The data was shown in mean + standard error.

Table 4. The correlation coefficient of sugar content and enzyme activities

	Sucrose	Fructose	Glucose	SPS	SPP
Sucrose	1	- 0.4498*	- 0.4279*	- 0.2283ns	0.7928***
Fructose		1	0.9474***	- 0.4203*	- 0.3512*
Glucose			1	- 0.4191*	- 0.2873ns
SPS				1	- 0.4534**
SPP					1

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