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ACTIVITY OF SUCROSE-PHOSPHATE SYNTHASE IN SUGAR CANE LEAVES

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Key Word Index—*Saccharum* sp.; Gramineae; sugar cane; biosynthesis; regulation; sucrose phosphate; sucrose.

Abstract—The activity of sucrose-phosphate synthase of sugar cane leaves was determined in response to reports that in sugar cane, sucrose synthase might be more important for sucrose synthesis than sucrose-phosphate synthase. The experiments revealed that there is sufficient sucrose-phosphate synthase activity to account for the *in vivo* rate of sucrose production in leaves, whereas sucrose synthase activity is less than 10% of that rate. The activity varied by a factor of two for leaves of different positions on the stalk. The sucrose-phosphate synthase of sugar cane leaves was stimulated three to four fold within 10 min above a threshold level by light and inactivated by darkness. Fructose-6-phosphate is an absolutely required substrate for sucrose-phosphate formation, in contrast to the tonoplast-bound group translocator in the stalk of sugar cane.

INTRODUCTION

Sucrose-phosphate synthase (UDP-D-glucose: D-fructose-6-phosphate 2α-D-glucosyl-transferase) (SPS) is generally accepted to be the enzyme responsible for sucrose synthesis in leaves [1]. Sucrose synthase (UDP-D-glucose: D-fructose 2α-D-glucosyl-transferase) (SS) is believed to be a degradative enzyme providing UDP-glucose in the cell for metabolic requirements. Little is presently known about the formation and turnover of sucrose in sugar cane leaves. Until now the emphasis in this plant has been on fate of sucrose in the stalk sink tissue rather than in its formation in the leaf source, although recently there has been a comparison of SPS and SS activity in sugar cane leaves [2] leading to the conclusion that sucrose is mostly formed by SS. It seemed appropriate to undertake a careful determination of SPS activity especially since the enzyme is reported in other plant species to be regulated by light [3, 4], a factor which was not considered in previous investigations. An additional interesting aspect is seen in the role of sucrose synthesis in connection with sucrose compartmentation. In mesophyll of barley, sucrose synthesized in the cytoplasm is transferred into the vacuole by a facilitated diffusion type transport system, so that the vacuole serves as a buffer between assimilate production and assimilate translocation to the phloem [5, 6]. For sugar cane parenchyma cells a totally different mechanism was reported, namely a tonoplast-bound enzyme complex which uses UDP-glucose to synthesize sucrose-phosphate in the vacuole with UDP-glucose as the sole substrate; neither fructose-phosphate nor cytoplasmic sucrose are direct precursor of vacuolar sucrose [7, 8]. It is interesting whether such a mechanism of sucrose synthesis also happens in sugar cane mesophyll cells, accomplishing vectorial sucrose synthesis into mesophyll vacuoles without interference of a soluble, fructose requiring SPS, which would have consequences on how to conceive the export of sucrose from mesophyll to phloem.

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RESULTS AND DISCUSSION

Rate of sucrose synthesis in sugar cane leaves

In vivo rates of sucrose synthesis are an essential prerequisite for evaluation of subsequent *in vitro* measurements of sucrose synthesizing enzymes. During daylight hours sugar cane leaves increase their sucrose content from 5 μmol/g fr. wt to 25 μmol/g fr. wt; the maximum increase is *ca* 4 μmol/hr/g fr. wt in the late morning hours (Fig. 1). In addition, some sucrose is translocated by the

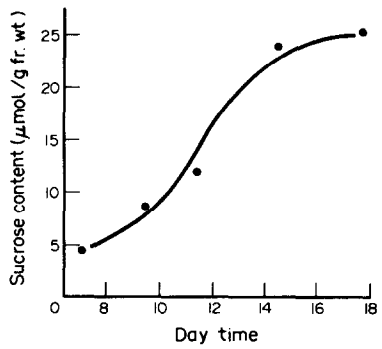

Fig. 1. Sucrose content of sugar cane leaf during the course of a day. Leaves of position 5 were harvested in the field and immediately quenched in 80% ethanol. They were extracted by incubation in ethanol for 5 hr at room temperature, the ethanolic solution was evaporated to dryness, dissolved in water, and sucrose was measured by the procedure of ref. [11]. Sunrise was at 7.00, sunset at 17.30.

phloem to the stem, which can be estimated to be *ca* 4 μmol/hr/g fr. wt (Hartt *et al.*, [9], showed that 63% of labelled sucrose was translocated within 90 min). Total sucrose synthesis, should therefore be *ca* 8 μmol/hr/g fr. wt or 2.2 nkat/g fr. wt. Any enzyme activity measured *in vitro* must approach that rate. Activities reported by Batta and Singh [2] for SPS are 40 pkat/g fr. wt and for sucrose synthase 120 pkat/g fr. wt thus, one order of magnitude below the minimal acceptable number.

Sucrose-phosphate synthase and sucrose synthase activity of sugar cane leaves

In order to obtain optimal activity, extraction of the enzymes was performed as rapidly as possible. Using field-grown leaves leaf pieces were plunged into liquid N_2 at time of excision and were extracted in buffer. The extract was centrifuged at low speed so that it still contained the membrane vesicles and was assayed for SPS and SS activity. Within the assay time of 30 min, the formation of sucrose was usually linear (Fig. 2), but nevertheless in each case the time-course of sucrose synthesis was followed. The formation of sucrose was parallel to the consumption of UDP-glucose, with no sucrose formed in the absence of UDP-glucose (Fig. 2). Though the crude extract was used, there were no other UDP-glucose consuming reactions besides sucrose synthesis (Fig. 3). Glucose arises partly from radioactively labelled UDP-glucose and is partly produced by the phosphatase treatment. Invertase in the raw extract obviously did not interfere with the assay, since the level of endogenous sucrose present in the extract hardly decreased within the assay period (Fig. 2).

The measured enzymatic activities were 29.9 nkat/g fr. wt for SPS and 2.7 nkat/g fr. wt for SS, suggesting the dominant role of SPS for sucrose synthesis. Assuming that the *in vivo* substrate concentrations (UDP-glucose and fructose-6-phosphate) are likely to be close to 1 mM instead of 8 mM, (as used in the *in vitro* assay) and taking the K_m of 4 mM for each substrate, in both enzymes [1] the enzymatic activity of SPS and SS can be expected to be one-tenth of that found under the assay conditions. This would be a value close to the observed *in vivo* rate

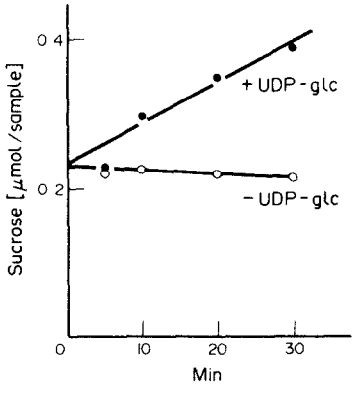

Fig. 2. Time-course of sucrose formation by sugar cane leaf extract in the presence and absence of UDP-glucose. Each time point (sample) was obtained by the standard experimental procedure with 35 μl leaf extract in 210 μl incubation medium.

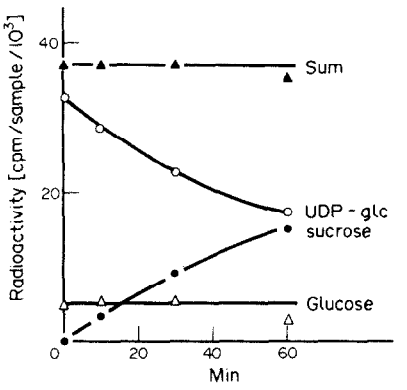

Fig. 3. Time-course of UDP-glucose consumption and sucrose formation by sugar cane leaf extract. Each time point was obtained by the standard experimental procedure, including 35 μl leaf extract and 900 Bq UDP-[^{14}C]glucose (corresponding to 38×10^3 cpm) in 210 μl medium. The reaction was stopped by heating for 10 min at 100°, the sample treated with alkaline phosphatase and subjected to PC.

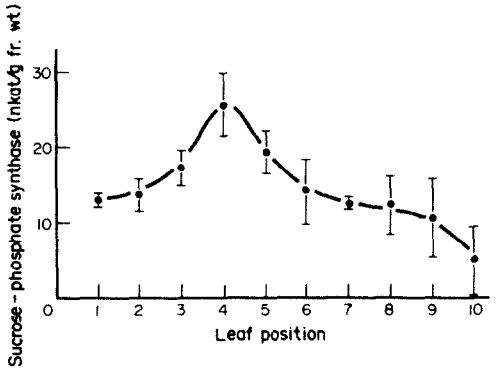

Fig. 4. Sucrose-phosphate synthase activity in sugar cane leaves of different ages. Leaf designated as 1 is the uppermost partially unrolled leaf, leaf number 2 is the next downward, etc.; leaf 4 is usually the leaf with the first visible dewlap. Normally there are 10–15 green unfolded leaves per stalk. Bars indicate standard deviation of the mean value.

(2.2 nkat/g fr. wt) in the case of SPS. The comparison of *in vitro* enzyme activities with *in vivo* rates relies on appropriate assay conditions. In this case the assay pH (7.5) was close to the expected cytoplasmic pH and the substrate concentrations were close to the V_{max} of the enzyme. It is not certain whether a differential inactivation of SPS and SS activity occurred during the extraction procedure. Sugar cane leaf extract causes some inactivation of spinach and wheat germ SPS and SS where SPS was inactivated more than SS. Therefore, it is probable that SPS is underestimated more than SS in sugar cane leaf extracts.

SPS activity is highest in the leaf with the first visible dewlap (the fourth leaf below the first partially unrolled leaf) and declines to half the rate towards younger and older leaves (Fig. 4). The same leaf has been reported to give the highest photosynthetic rate [10].

Stimulation of sucrose-phosphate synthase by light

It has been previously shown that SPS activity of barley, soybean, and maize [3, 4] increases when leaves are exposed to light. Similar results could be obtained with sugar cane leaf SPS. When leaves of field-grown plants were darkened by wrapping them with aluminum foil, SPS activity decreased to one-third of the activity of the leaves exposed to sun light (Fig. 5A); 10 min of dark treatment decreased SPS activity nearly 50%. Removing the aluminum foil and reexposing the leaves to sunlight gave a rapid increase of SPS activity in the test system, a recovery of activity amounting to *ca* 50% was obtained after 10 min illumination.

The decrease in leaf sucrose concentration in darkness only partially reflected the rapid drop in SPS activity, and sucrose was also less responsive than SPS when full sunlight conditions were restored (Fig. 5B).

Properties of sucrose-phosphate synthase of sugar cane leaves

It seems to be very difficult to preserve SPS activity in sugar cane leaf extracts; the presence of 1% polyvinylpyrrolidone and 10 mM dithiothreitol was required to achieve stability for a test period of 30 min. Substrates aided stability, but even then SPS activity was completely lost within 18 hr. (Protease inhibitors (phenylmethysulphonylfluoride and aprotinin) did not improve stability; fractionation with ammonium sulphate precipitation inactivated the enzyme). Precautions and the optimization of SPS activity under full sunlight conditions may account for discrepancies between our and other investigators results [2].

The SPS of sugar cane leaves has a strict requirement for fructose-6-phosphate with a K_m of *ca* 7 mM (Fig. 6), in contrast to the tonoplast-bound enzyme complex which was reported to form sucrose in sugar cane stalk cells [8]. UDP-Glucose is used by the leaf SPS with a K_m of *ca* 4 mM. Since the leaf extract used in the present experiments included all microsomal membranes, it seems that sugar cane does not contain a membrane-bound UDP-glucose group translocator to synthesize sucrose. Sucrose synthesis in sugar cane leaves obviously follows the same pathway as in leaves of other plant species [1].

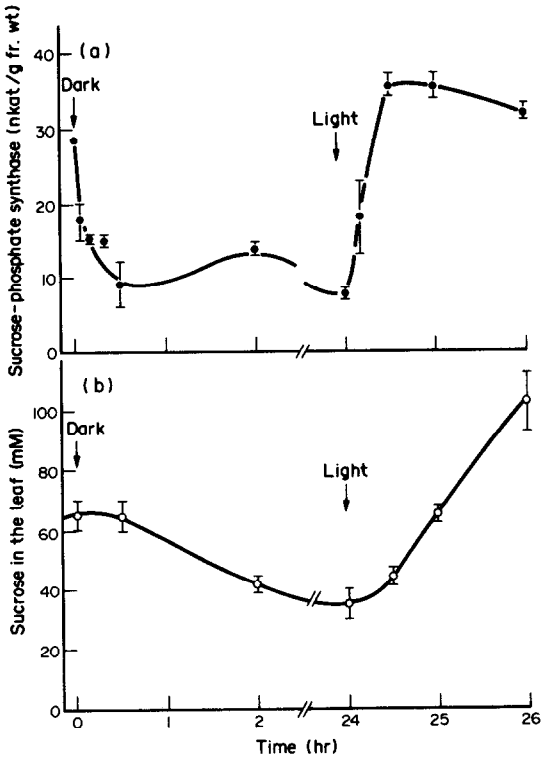

Fig. 5. Effect of light on the activity of sucrose-phosphate synthase (A) and the sucrose content (B) of sugar cane leaves. Leaves showing the first visible dewlap (number 4 or 5) were covered at 10 a.m. with aluminum foil. The activity of SPS in the leaf extract was determined at periodic intervals over the next 24 hr. Leaves covered for 24 hr with aluminum foil were uncovered at 10 a.m., harvested and tested for SPS at different times after sunlight exposure. Bars indicate the standard deviation of the means of three replicates.

EXPERIMENTAL

Leaf sampling and extraction. Leaves of sugar cane (*Saccharum* sp. L., hybrid H 65-7052) were harvested in the field at 9 am in full sunlight. The leaf with the first visible dewlap (the 4th leaf, counting from the top with the first partially unrolled lamina as number one) was cut into 3 cm pieces, which were plunged into liquid N_2. The tip and the part near the sheath were not used. Frozen leaf pieces were ground in liquid N_2 in the presence of washed sand and PVP (40 mg/g leaf fr. wt). The ground tissue was transferred to an ice-cold mortar and grinding was continued in extraction buffer (40 mM HEPES pH 7.5, 15 mM $MgCl_2$, 10 mM dithiothreitol and 2% PEG M_r 20,000), in 4 ml of extraction buffer/g leaf fr. wt. The suspension was centrifuged at 400 *g* for 10 min at 2° and the supernatant dialysed for 15 min at 4° against extraction buffer.

Determination of enzyme activity. SPS was measured by a modification of the method of ref. [4]. Determination of sucrose-phosphate was carried out in 210 μl samples containing 8 mM UDP-glucose, 8 mM fructose-6-phosphate, 15 mM Mg Cl_2 and 40 mM HEPES pH 7.5 and 35 μl of dialysed leaf ext (corresponding to *ca* 60 μg protein). The soln was incubated at 37°, quenched by addition of 70 μl of 1 N NaOH and boiled for

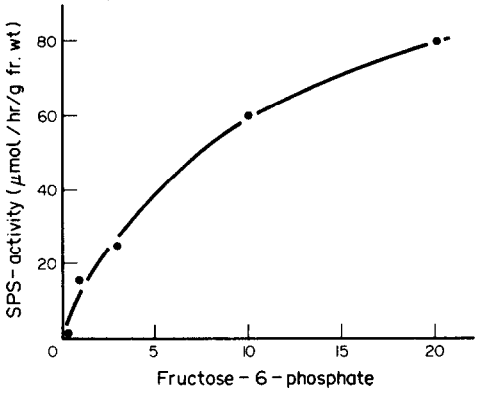

Fig. 6. Concentration dependence of sucrose synthesis on fructose-6-phosphate.

10 min. After cooling to 15°, 0.5 ml H_2O, 0.25 ml resorcinol (1% EtOH soln) and 1.75 ml HCl (30%) were added and heated at 80° for 8 min. After cooling to room temp the *A* at 520 nm was determined [11].

For some expts the reaction was measured via generation of UDP [12], or by the use of UDP-[^{14}C] glucose. In the latter case the incubation of the sample was terminated by heating. Alkaline phosphatase was added, the mixture incubated for 20 min at 37° and the sample transferred to an anion exchange column (Bio-Rad AG 1- × 4- Cl). The eluted labelled sucrose was determined by liquid scintillation counting.

In all cases the time course of enzyme activity was measured with 5 samples over a 30 min incubation period. Controls were performed with the same procedure but lacking either UDP-glucose or leaf ext.

SS was determined in the same manner using a similar procedure to SPS, substituting fructose for fructose-6-phosphate.

Spinach SPS was prepd and assayed according to ref. [13], wheat germ SS was obtained from Sigma. The reaction products were sepd by PC in *n*-BuOH–HOAc–H_2O (4:1:5, upper phase). Samples were subjected to alkaline phosphatase treatment prior to chromatography.

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