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IN VITRO AND *IN VIVO* INHIBITION OF SUCROSE SYNTHESIS BY SUCROSE*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; sucrose phosphate synthase; sucrose synthesis; sucrose inhibition.

Abstract—The inhibitory effects of sucrose on rates of sucrose synthesis by sucrose phosphate synthase (SPS) from the maize scutellum and on net rates of sucrose production in maize scutellum slices from added glucose or fructose were studied. Scutellum extracts were prepared by freezing and thawing scutellum slices in buffer. The extracts contained SPS and sucrose phosphate phosphatase, but were free of sucrose synthase. SPS activity was calculated from measurement of UDP formation in the presence of UDPG, fructose-6-P and sucrose. The ranges of metabolite concentrations used were those estimated to be in scutellum slices after incubation in water or fructose for periods up to 5 hr. UDPG and fructose-6-P also were added at concentrations that saturated SPS. At saturating substrate levels, sucrose inhibition of SPS was less than that when tissue levels of substrates were used. With tissue levels of substrates and sucrose concentrations up to *ca* 166 mM, sucrose inhibitions of sucrose synthesis *in vitro* by SPS were similar to those observed *in vivo*. However, as the sucrose concentration rose above 166 mM, SPS activity was not inhibited further, whereas there was a further sharp decline in sucrose production by the slices. It is concluded that sucrose synthesis *in vivo* is controlled by sucrose inhibition of SPS over a considerable range of internal sucrose concentrations.

INTRODUCTION

In germinating cereal grains, the products of starch hydrolysis in the endosperm enter the scutellum and are converted to sucrose [1, 2]. When maize scutellum slices are incubated in glucose or fructose, sucrose is synthesized and accumulated at rates that decrease as the sucrose content of the tissue increases [3]. Reduced rates of sucrose synthesis also occur in rice leaves with increasing internal sucrose concentration [4], and in sugar cane internode tissue as it matures and sucrose is accumulated to high levels [5, 6]. It appears that the rate of sucrose synthesis may be controlled by the concentration of tissue sucrose.

Synthesis of sucrose in higher plants is thought to occur primarily by the sucrose phosphate synthase pathway [7, 8]:

$$\text{UDPG} + \text{fructose-6-P} \longrightarrow \text{UDP} + \text{sucrose phosphate} \quad (1)$$

$$\text{Sucrose phosphate} + H_2O \longrightarrow \text{sucrose} + P_i \quad (2)$$

Reaction (1) is catalysed by sucrose phosphate synthase (SPS) which, when coupled to sucrose phosphate phosphatase [SPP, reaction (2)], forms an irreversible system. SPS preparations from wheat germ [9] and from leaves of tobacco, pea, bean and peanut [10] have been shown to be inhibited by sucrose.

The question remains whether sucrose inhibition of SPS explains the apparent sucrose inhibition of sucrose synthesis *in vivo*. In this study, the effects of sucrose concentration on sucrose synthesis *in vivo* and on SPS activity *in vitro* were investigated. The results indicate that over a considerable range of internal sucrose concentrations sucrose synthesis in the maize scutellum is controlled by sucrose inhibition of SPS.

RESULTS AND DISCUSSION

Sucrose synthesis in vivo

The internal sucrose content of maize scutellum cells was experimentally manipulated to study the *in vivo* effect of sucrose on sucrose synthesis. Slices were incubated in water to reduce the sucrose content, or in fructose or glucose to increase the sucrose content. During a 5 hr incubation in water, the sucrose content decreased from *ca* 33 to 17 μmol/g (cf. [11]). Longer incubation in water or separation of the embryo from the endosperm 24 hr before harvesting the scutella resulted in some browning and such slices were not used.

When slices were incubated in 100 mM fructose or glucose for 5 hr, the sucrose content increased to 85–105 μmol/g, and the rate of sucrose increase declined with incubation time (cf. [3]). It is assumed that sucrose accumulation is equivalent to sucrose synthesis in the slices (i.e. that no sucrose is lost by catabolism or leakage). This assumption is based on the following considerations: (a) sucrose leakage from the slices (measured during incubation in fructose) was negligible; (b) the maize

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cultivar used in these experiments contained a cell-wall invertase, but no cytoplasmic or vacuolar invertase [11]; (c) glucose strongly inhibits sucrose synthase (SS) in the breakdown direction ([12]; Echeverria, E. and Humphreys, T., unpublished results).

Sucrose accumulation during incubation of the slices in glucose is assumed to reflect the activity of SPS because the cytoplasmic fructose concentration was probably too low (the scutellum plasmalemma is freely permeable to fructose [13]) to support significant rates of sucrose synthesis by SS. Rates of sucrose accumulation in fructose were 10–20% greater than those in glucose, and this probably resulted from the action of SS in the synthesis direction.

In order to compare *in vivo* and *in vitro* effects of sucrose, fructose-6-P and UDPG on rates of sucrose synthesis, it was necessary to express tissue levels (μmol/g) of these compounds as concentrations (see Experimental). It was assumed that sucrose was uniformly distributed in the cell water although in some other tissues (e.g. *Tulipa* petals and sugar cane suspension cells) this appears not to be the case [14, 15]. However, these tissues accumulate hexose and contain cytosolic invertase, whereas scutellum cells do not. It was also assumed that fructose-6-P and UDPG were confined to the cytosol, i.e. that the amounts in the proplastids were negligible (cf. [16]).

Sucrose synthesis in vitro

The crude extracts used to measure SPS activity contained SPP activity, were free of invertase activity, and did not degrade UDPG in the absence of fructose and fructose-6-P (F-6-P). In addition, extract II (see Experimental) was free of SS activity. The activity of SPS was followed by measuring the formation of UDP at pH 8.0 (the estimated pH of the scutellum cytoplasm [17]), with substrates at either saturating or tissue levels. The synthesis of sucrose was monitored in some experiments, and the amount of sucrose was found to be equal to that of UDP.

SPS activity as a function of F-6-P and of UDPG concentration is shown in Fig. 1. Cellular F-6-P concentration reached saturating levels for SPS activity during the first hour of incubation of scutellum slices in fructose, whereas the UDPG concentration did not reach saturating levels even after 5 hr (Fig. 2).

The rate of UDP formation was constant for at least the first 15 min at saturating levels of UDPG and F-6-P (Fig. 3). The reaction mixture contained sodium fluoride and EDTA, and sucrose phosphate accumulated in their presence. Accumulation of sucrose phosphate was indicated by the absence of sucrose in the reaction mixture, but alkaline phosphatase (Sigma type I) caused the release of sucrose in amounts equal to the UDP formed. The calculated concentration of both sucrose phosphate and UDP at the end of the 15 min reaction period in Fig. 3 was 4.1 mM. Since the rate was constant, it appears that sucrose phosphate and UDP had no influence on the activity of SPS under the experimental conditions.

Extract I, obtained by use of a Virtis homogenizer, contained the greatest SPS activity. In the presence

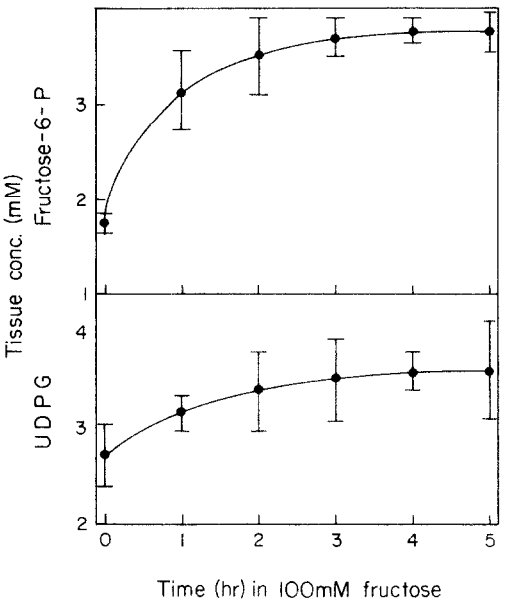

Fig. 2. Concentrations of F-6-P and UDPG in scutellum slices with time of incubation in fructose. Data are averages from three experiments.

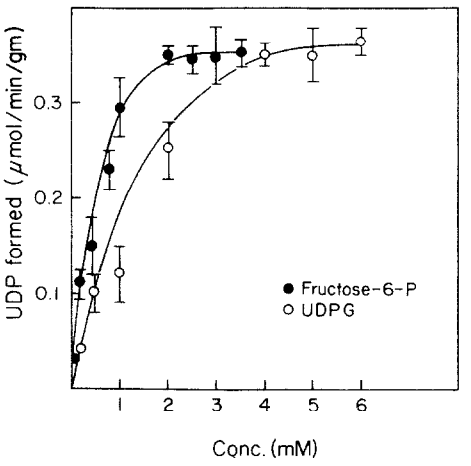

Fig. 1. Effect of UDPG and F-6-P concentrations on SPS activity in extract II. Data are averages from three experiments.

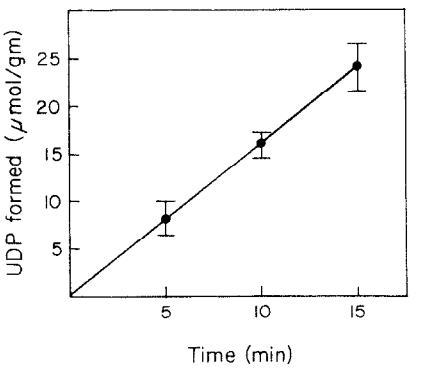

Fig. 3. Activity of SPS with incubation time at saturating levels of UDPG and F-6-P. Data are averages from five experiments.

of 20 mM β-phenylglucoside (needed to inhibit SS), the SPS activity (V_{max}) of five preparations of extract I was 1.63 ± 0.13 μmol UDP formed/min per g fr. wt of slices. Because sucrose competes with β-phenylglucoside for SS [12], β-phenylglucoside would not be suitable for experiments testing the effects of sucrose on SPS. Therefore, extract II, obtained by freezing and thawing scutellum slices, was used to measure sucrose inhibition of SPS because it contained no SS activity. The SPS activities (V_{max}) of preparations of extract II varied from 0.3 to 0.4 μmol UDP formed/min, only 18–25% of the activity of extract I.

In order to compare *in vitro* SPS activities with *in vivo* rates of sucrose synthesis, it was necessary to multiply the SPS activities of extract II by the factor 1.63/0.3 to 0.4, i.e. by the ratio of the V_{max}s for SPS in the two extracts. Because V_{max} for SPS in extract II was variable, the value of the factor was different for each preparation of extract II. The assumption made in using this factor is that extract I contained all the SPS activity of the tissue.

The inhibitory effect of sucrose on SPS activity at saturating levels of UDPG and F-6-P is shown in Fig. 4. SPS activity decreased sharply at sucrose concentrations up to 20 mM and less sharply as the sucrose concentration increased above 20 mM. A maximum inhibition of 51% was obtained at 200 mM sucrose. SPS was not inhibited by other disaccharides, by mono- and trisaccharides, or by hexitols.

SPP is also inhibited by sucrose [8, 18]. SPP from the maize scutellum was inhibited 73% by 100 mM sucrose [18]. However, maize scutellum extracts contain 30–100 times as much SPP activity as SPS activity, which indicates that sucrose phosphate would not accumulate in cells during sucrose synthesis [18].

Sucrose inhibition of SPS at cellular levels of UDPG and F-6-P

The *in vivo* rate of sucrose synthesis declined as the sucrose concentration of the slices increased (Fig. 4), and it is presumed that this was caused by sucrose inhibition of SPS. The apparent *in vivo* inhibition was greater than the *in vitro* inhibition of SPS. However, the levels of F-6-P and UDPG in the cells were initially below levels that saturate SPS (Figs. 1 and 2). When SPS was tested at F-6-P and UDPG concentrations found in the slices at zero time, inhibition by sucrose was increased (Fig. 4) and was similar to the inhibition obtained *in vivo*, if it is assumed that the dashed line in Fig. 4 gives the correct *in vivo* rate of sucrose synthesis at zero sucrose concentration.

SPS activity was tested using concentrations of sucrose and substrates found in slices at zero time and after each hour of a 5 hr incubation in fructose (cf. Fig. 2). With concentrations found at zero time, SPS was inhibited *ca* 60% (Figs. 4 and 5), and *in vitro* and *in vivo* rates of sucrose synthesis were similar (Fig. 5). However, as the sucrose concentration increased above 58 mM, SPS activity did not change significantly, whereas the *in vivo* rate of sucrose synthesis continued to decline (*ca* 25% between 58 and 166 mM). The sharp decline of sucrose synthesis when sucrose concentrations rose above 166 mM (Fig. 5) could be due to factors that only indirectly affect SPS, such as the effect of a lowered cytosolic water potential on mitochondrial function. However, such high concentrations would not be expected in the scutellum of an

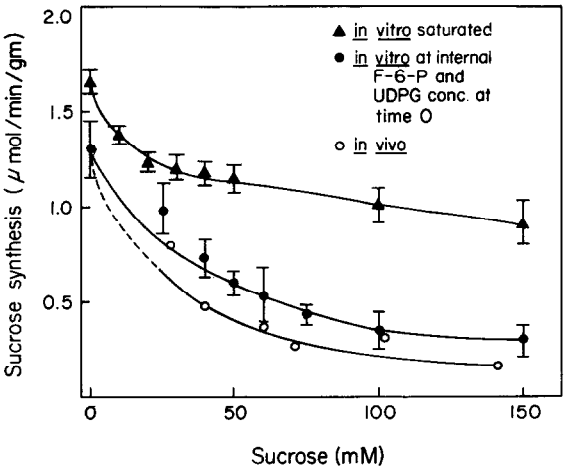

Fig. 4. Effect of sucrose concentration on SPS activity and on sucrose production in scutellum slices. SPS activity was measured at saturating levels of UDPG and F-6-P or at concentrations of these substrates estimated to be in the slices at the start of the fructose incubation. Each point of the SPS curves is an average from three or more experiments. The *in vivo* results are averages from three to five experiments, and standard deviations were less than 20% of the means.

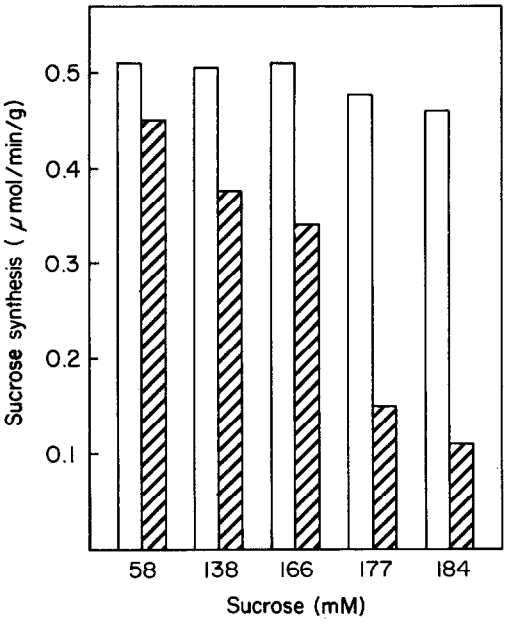

Fig. 5. *In vivo* and *in vitro* rates of sucrose synthesis at five sucrose concentrations. Slices were incubated in 100 mM fructose, and the levels of UDPG, F-6-P and sucrose were determined at zero time and at the end of each hour of a 5 hr period (results from the first hour are not shown). Tissue concentrations were estimated from the tissue levels of these compounds using the assumptions discussed in the text. Rates of *in vivo* sucrose synthesis (hatched bars) were calculated. Rates of *in vitro* sucrose synthesis (open bars) by SPS were determined using those concentrations of UDPG, F-6-P and sucrose estimated to be in the slices. Data are averages from four experiments. Standard deviations were less than 10% of the means (less than 20% for the hatched bars at 177 and 184 mM).

intact seedling because of sucrose export to the root and shoot.

The sucrose concentration of the slices at zero time was *ca* 50 mM, high enough to inhibit SPS strongly (Fig. 4). Therefore, it appears that in the intact 3-day-old seedling the scutellum SPS is in the inhibited state and that the capacity for sucrose synthesis of the scutellum can accommodate the demands of older and larger seedlings. The results of Fig. 4 suggest that the rate of sucrose synthesis *in vivo* would be most sensitive to sucrose in the 0–40 mM range. Unfortunately, only part of this range could be obtained experimentally.

Conclusions

The following conclusions are drawn: (a) SPS is responsible for sucrose synthesis *in vivo* since similar *in vivo* rates of sucrose synthesis were obtained when scutellum slices were incubated in either glucose or fructose; (b) SPS is strongly inhibited *in vivo* by sucrose, even by sucrose levels found before incubation in hexose (Figs. 4 and 5); and (c) in the intact seedling, sucrose synthesis in the scutellum may be controlled by the sucrose concentration, rates of synthesis changing as the demand for sucrose by the root–shoot axis changes.

EXPERIMENTAL

Preparation of slices. Slices were prepared from scutella of 3-day-old maize seedlings (*Zea mays* L. cv DeKalb XL 80) as previously described [17].

Sucrose synthesis in scutellum slices. Groups of slices (0.5 mm or less in thickness, 0.5 g) were placed in 50 ml beakers, incubated in 10 ml H_2O for 30 min at 30° in a gyratory H_2O bath, and then incubated in a soln containing 10 mM each of $CaCl_2$, $MgCl_2$ and KCl for 30 min. Salt treatment reduced sucrose leakage during the subsequent incubation in hexose. At this time (time 0), the slices were washed once with H_2O and 9 ml of fructose or glucose were added. The hexose solns (100 mM) were replaced every 30 min until termination of the experiment. A set of three groups of slices was killed at time 0 and after each hour in hexose. Slices were killed by boiling in 80% EtOH for 30 sec. After 1 hr at room temp., the EtOH soln was decanted, and the slices were washed with 80% EtOH (3 × 5 ml portions). The combined extracts were evapd on a steam bath to a vol. of *ca* 2 ml, and transferred to a graduated tube. The vol. was adjusted to 25 ml with 5 ml Mops (100 mM, pH 7.5), 3 ml 0.3 M $MgCl_2$ and H_2O. After centrifugation (1000 *g*, 10 min), the soln was analysed for sucrose and/or F-6-P. Rates of sucrose synthesis were calculated from increases in the average sucrose content of a set of three groups of slices following each hour of incubation in fructose (Figs. 4 and 5). Sucrose synthesis is expressed as μmol sucrose formed per min in 1 g of slices (μmol/min per g fr. wt).

Determination of sucrose and F-6-P. Samples for sucrose determination were incubated with and without invertase (Sigma Grade X) prior to glucose analysis using glucose oxidase. F-6-P was assayed by the procedure described in ref. [19].

Determination of UDPG. Slices (0.5 g) were killed in 5 ml boiling Epps (50 mM, pH 8.0). After cooling for 30 min, 5 ml 50 mM $MgSO_4$ was added, the soln was centrifuged (1000 *g*, 10 min), and the supernatant fraction was filtered through Whatman No. 43 paper. Aliquots of 2 ml were combined with 1 ml Tris–HCl (50 mM, pH 7.5) and 1 ml 1 mM $MgCl_2$, and analysed for UDPG with UDPG dehydrogenase [19]. When known amounts of UDPG were added before killing the slices, recovery was 96% or greater.

Calculation of internal concns of sucrose, F-6-P and UDPG. The slices contained 70% H_2O (350 μl/0.5 g). In calculating sucrose concns, it was estimated that 80% (280 μl) of the tissue H_2O was inside the plasmalemma, and it was assumed that sucrose was uniformly distributed in this vol. The 80% value was arrived at from estimates of wall and intercellular spaces using photomicrographs. It was assumed that UDPG and F-6-P were uniformly distributed in the cytosol, whose vol. was assumed to be the same as the glucose space (40 μl/0.5 g or 14.3% of the vol. inside the plasmalemma [17]). To convert the tissue concns given in the figures to μmol/g, multiply by 0.56 (sucrose) or by 0.08 (UDPG and F-6-P).

Preparation of scutellum enzyme extracts. Two methods were used. The first method gave maximum recovery of SPS. To prepare extract I, slices (*ca* 1.5 mm in thickness, 10 g) were homogenized in a Virtis '60 K' homogenizer for 2 min in 100 ml Tris–HCl (100 mM, pH 8.7) containing 6 mM cysteine–HCl and 4 mM EDTA. The homogenate was strained through cheesecloth and centrifuged in the cold (21 000 *g*, 20 min). The supernatant fraction was dialysed in 4 l. Tris–HCl (5 mM, pH 8.7) overnight. Extract I was used in the experiments of Fig. 3.

To prepare extract II, slices (*ca* 1.5 mm in thickness, 5 g) were placed in beakers and frozen overnight. Freezing and thawing eliminates interference by SS. The tissue was thawed at 30° for 30 min in 10 ml Tris–HCl (100 mM, pH 8.7), 6 mM cysteine–HCl and 4 mM EDTA. The soln was decanted and dialysed overnight against 4 l. Tris–HCl (5 mM, pH 8.7). Extract II was used in the experiments of Figs. 1, 4 and 5.

SPS assay. The reaction mixture (0.5 ml) for SPS assay contained Epps (80 mM, pH 8.0), 20 mM EDTA, 10 mM NaF, various concns of UDPG, F-6-P, G-6-P and sucrose and scutellum extract. When SPS activity was measured under conditions of substrate saturation, the concns of UDPG and F-6-P were 15 mM. Because the extracts contained phosphohexose isomerase, enough G-6-P was added so that the ratio F-6-P/G-6-P was 3:7. NaF and EDTA were added to inhibit phosphatases. When extract I was used, sucrose was omitted and 20 mM β-phenylglucoside was added to inhibit SS [12]. Reactions were carried out at 30° for 5–15 min, and terminated by placing the reaction tube in boiling H_2O for 2 min. After centrifugation (1000 *g*, 10 min), portions of the supernatant fraction were analysed for UDP by the procedure described in ref. [19]. Sucrose synthesis by SPS is expressed as μmol UDP formed per min by the SPS from 1 g of slices (μmol/min per g fr. wt of slices).

REFERENCES

1. Edelman, J., Shibko, S. and Keys, A. (1959) *J. Exp. Botany* **10**, 178.
2. Nomura, T., Kono, Y. and Akazawa, T. (1969) *Plant Physiol.* **44**, 765.
3. Humphreys, T. (1977) *Phytochemistry* **16**, 1359.
4. Tschen, J., Chen, P. and Liu, H. (1979) *Plant Protection Bull.* **21**, 305.
5. Bieleski, R. (1960) *Aust. J. Biol. Sci.* **13**, 203.
6. Glasziou, K. (1960) *Plant Physiol.* **35**, 895.
7. Leloir, L. and Cardini, C. (1955) *J. Biol. Chem.* **214**, 157.
8. Hawker, J. and Hatch, M. (1966) *Biochem. J.* **99**, 102.
9. Salerno, G. and Pontis, H. (1978) *FEBS Letters* **86**, 263.
10. Huber, S. (1981) *Z. Pflanzenphysiol.* **102**, 443.
11. Echeverria, E. and Humphreys, T. (1984) *Phytochemistry* **23**, 2173.
12. Wolosiuk, R. and Pontis, H. (1974) *Mol. Cell Biochem.* **4**, 115.
13. Humphreys, T. (1974) *Phytochemistry* **13**, 2387.
14. Wagner, G. (1979) *Plant Physiol.* **64**, 88.

15. Thom, M., Maretzki, A. and Komor, E. (1982) *Plant Physiol.* **69**, 1315.
16. Dennis, D. and Miernyk, J. (1982) *Annu. Rev. Plant Physiol.* **33**, 27.
17. Humphreys, T. (1982) *Phytochemistry* **21**, 2165.
18. Hawker, J. (1971) *Phytochemistry* **10**, 2313.
19. Bergmeyer, H.-U. (1965) *Methods of Enzymatic Analysis.* Academic Press, New York.