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GLUCOSE CONTROL OF SUCROSE SYNTHASE IN THE MAIZE SCUTELLUM*

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

Abstract—The *in vivo* amounts of UDPG, UTP, UDP and UMP, metabolites known to influence the activity of sucrose phosphate synthase (SPS) and sucrose synthase (SS), were measured throughout 5 hr incubations of scutellum slices in fructose or water, i.e. under conditions of sucrose synthesis or breakdown. Cytosolic concentrations were estimated assuming that these metabolites were confined to the cytosol. Within the estimated *in vivo* concentration ranges, UDPG, UTP and UDP had little effect on the *in vitro* SS activity, but glucose (100 mM) inhibited SS in the synthesis direction by 63–70% and in the breakdown direction by 86–93%. Glucose inhibition of SS was considerably less when saturating levels of substrates were used. Sucrose did not inhibit SS. It is concluded that during germination the glucose produced from starch breakdown in the maize endosperm enters the scutellum and inhibits SS, preventing a futile cycle and limiting SS participation in sucrose synthesis.

INTRODUCTION

There are two mechanisms for sucrose synthesis in higher plants [1, 2]. It is thought that the role of sucrose synthase (SS) is to break down sucrose and form nucleoside diphosphoglucose, since it catalyses a readily reversible reaction; while the role of the sucrose phosphate synthase–sucrose phosphate phosphatase (SPS–SPP) complex is to synthesize sucrose [3–6]. However, a synthetic role for SS cannot be ruled out as demonstrated by Gross and Pharr [7] who showed that the galactosyl moiety of stachyose is converted to sucrose in *Cucurbita* peduncles although this organ contains SS but not SPS.

During germination of cereal grains, the products of the breakdown of endosperm starch (mainly glucose and maltose) are converted to sucrose in the scutellum, and the sucrose is transported to the growing axis [8, 9]. Slices of maize scutellum incubated in glucose or fructose synthesize sucrose, and rates of sucrose synthesis decline as the internal sucrose content increases [10]. Both SS and SPS have been found in the maize scutellum [11–13], and the questions arise as to which enzyme is involved in sucrose synthesis and as to how SS is controlled in the breakdown direction.

Sucrose synthesis by SPS followed by sucrose breakdown by SS would constitute a futile cycle [14]. This paper reports an investigation of some factors regulating the synthesis and breakdown of sucrose in the maize scutellum that might prevent such a futile cycle.

RESULTS AND DISCUSSION

Cellular concentration of metabolites

Rates of sucrose synthesis and breakdown might be influenced by the cytosolic concentrations of UDPG, UTP, UDP and UMP, and therefore, these metabolites were measured in scutellum slices incubated in water or fructose, i.e. under conditions of sucrose breakdown or synthesis. In order to compare the *in vivo* and *in vitro* effects of the uridine compounds on SS and SPS activities, it was necessary to express tissue levels (μmol/g) of these compounds as concentrations (see Experimental). It was assumed that the uridine compounds were confined to the cytosol, i.e. that the amounts in the organelles were negligible.

When slices were incubated in 100 mM fructose for 5 hr, the UDPG concentration increased from 2.7 mM to 3.7 mM, and the concentrations of UTP, UDP and UMP decreased slightly from their initial values (Fig. 1A). The sum of the concentrations of the four uridine compounds was constant during the 5 hr incubation period.

When slices were incubated in water for 5 hr, the UDPG concentration decreased from 2.7 mM to 1.7 mM, UTP concentration increased from 1.2 mM to 2.9 mM, and UDP and UMP increased to final concentrations of 1.0 mM and 0.9 mM (Fig. 1B). At the end of the 5 hr incubation period, the total amount of the four uridine compounds was 26% greater than it was at time zero. This is presumed to result from conversion of other uridine nucleotide sugars (e.g. UDP galactose) during the incubation in the absence of exogenous hexose.

Synthesis activity of sucrose synthase (SS)

The *in vivo* net rate of sucrose synthesis did not exceed 0.45 μmol/min per g fr. wt of slices during the 5 hr

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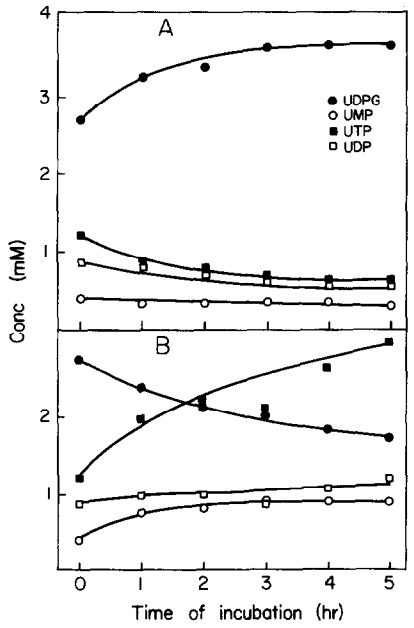

Fig. 1. Concentrations of uridine compounds in scutellum slices with time of incubation in 100 mM fructose, A; or in water, B. Each point is an average from three experiments, with an s.d. of less than 12%.

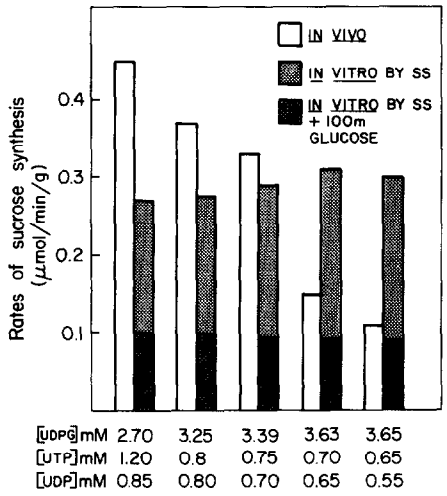

Fig. 2. *In vivo* rates of sucrose production by scutellum slices and *in vitro* synthesis activity of SS at UDPG, UTP and UDP concentrations found in the slices at each hour of a 5 hr incubation in 100 mM fructose. Each column is an average from two experiments.

incubation in fructose (Fig. 2). The maximum *in vitro* rate (V_{max}) for the amount of SS extracted from 1 g of scutellum slices was 0.39 μmol sucrose synthesized/min [12]. The K_ms for fructose and UDPG for maize scutellum SS were found to be 1.1 mM and 2.1 mM, respectively [unpublished results]. The tissue UDPG concentration was greater than the K_m (Fig. 1A), and since hexoses readily penetrate scutellum cells [15], fructose was undoubtedly at saturating levels inside the cells when 100 mM fructose was outside. From these data it is clear that SS could account for most or all of the *in vivo* sucrose synthesis (Fig. 2). The extent of SS involvement in sucrose synthesis is the subject of this section.

At saturating levels of fructose and UDPG, sucrose synthesis by SS was not inhibited by sucrose at concentrations up to 100 mM, whereas glucose inhibited the synthesis activity up to 35% at concentrations between 50 and 100 mM (Fig. 3). In contrast to these results, sucrose inhibits maize scutellum SPS and also inhibits sucrose synthesis in scutellum slices [13]. The insensitivity of SS to sucrose indicates that SS does not play a major role in sucrose synthesis in the maize scutellum. This is also indicated by the glucose inhibition of SS, since glucose entering from the endosperm is present in the scutellum cell during sucrose synthesis in the intact seed. Strong inhibition of SS by UDP only occurred at concentrations above 1 mM (Fig. 3) whereas tissue levels of UDP remained below 1 mM (Fig. 1A).

Activities of SS in the synthesis direction were measured in reaction mixtures containing UDPG, UTP and UDP at the concentrations estimated to be in the scutellum cells after each hr of incubation in 100 mM fructose (Fig. 1A). The reaction mixtures contained saturating levels of fructose in the absence or presence of 100 mM glucose. *In vitro* rates with SS were compared with *in vivo* net rates of sucrose synthesis obtained during a 5 hr incubation of slices in 100 mM fructose (Fig. 2).

Changes in UDPG, UTP and UDP within the concentration ranges estimated to be in the cytosol had little effect on the *in vitro* synthetic activity of SS. However, glucose inhibited SS activity *in vitro* by 63–70% when *in vivo* levels of uridine compounds were used (Fig. 2). *In vivo* rates of sucrose synthesis declined during the 5 hr incubation, the rate during the fifth hr being only about 25% of the rate during the first hr. The decline in the *in vivo* rate appears to be caused by the tissue sucrose concentration [13], which increased greatly during the 5 hr incubation (cf. [10]), and it was not due to changes in the levels of UDPG, UTP and UDP [13]. From these results, it appears that when the sucrose content of the scutellum is relatively low and the fructose and glucose contents are high, SS contributes no more than 22% of the sucrose synthesis activity (first set of bars, Fig. 2). However, in the intact seedling, the fructose concentration of the scutellum would probably be below saturation because the only fructose source would be fructose-P (the scutellum cells of the cultivar used in these experiments had no intracellular

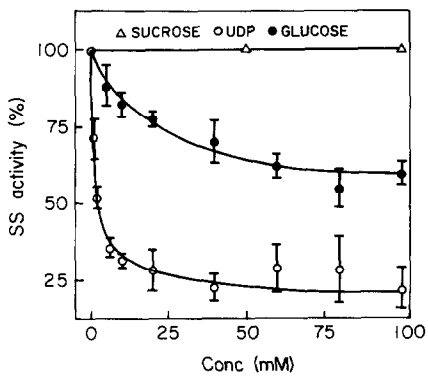

Fig. 3. Effects of sucrose, glucose and UDP on the synthesis activity of SS. Each point is an average from three experiments.

invertase [12]) and because fructose can readily diffuse to the cell exterior [15]. Therefore, SS would contribute still less to sucrose synthesis in the intact seedling.

Scutellum slices incubated in 100 mM fructose synthesized sucrose at rates 10–20% greater than those incubated in glucose [10]. During incubation in fructose, the substrates (UDPG, fructose-6-P and fructose) for both SPS and SS were at or near saturation levels (Fig. 1A, [13]), and presumably both enzymes participated in sucrose synthesis.

Breakdown activity of sucrose synthase

Previously, evidence was presented that indicates SS is responsible for sucrose breakdown in the maize scutellum [12], and it appears from the evidence presented above that SS plays only a small role in sucrose synthesis, being 'turned off' in the synthesis direction by glucose. To prevent a futile cycle, it is also necessary that SS be 'turned off' in the breakdown direction during sucrose synthesis.

The breakdown activity of SS was tested at concentrations of UDPG, UTP and UDP estimated to be in the cytosol after each hour of incubation of slices in water (Fig. 1B). Under these conditions SS activity was 75–85% of V_{max}, and was 3- to 20-fold greater than *in vivo* rates of sucrose breakdown (Fig. 4). Increasing UDP concentrations had little effect on breakdown activity (Fig. 4), probably because the enzyme was already near saturation (Fig. 5).

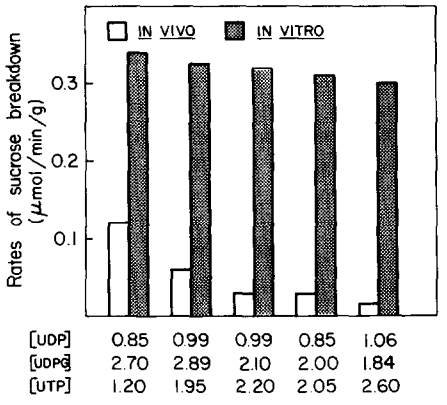

Fig. 4. *In vivo* rates of sucrose breakdown by scutellum slices and *in vitro* breakdown activity of SS at UDPG, UTP and UDP concentrations found in the slices at each hour of a 5 hr incubation in water. Each column is an average from two experiments.

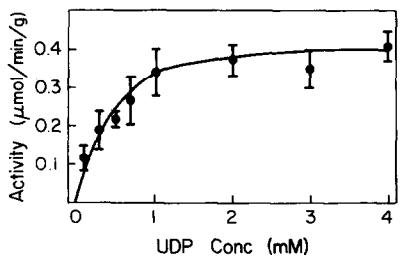

Fig. 5. Effect of UDP concentration on the breakdown activity of SS. Each point is an average from three experiments.

The difference between the *in vivo* and *in vitro* rates of sucrose breakdown (Fig. 4) might result from the cellular distribution of sucrose. The sucrose content of the slices decreased during the water incubation from *ca* 35 μmol/g initially to *ca* 20 μmol/g after 5 hr [12]. These quantities convert to 63 mM and 36 mM, assuming the sucrose was evenly distributed in the cell water. Since the K_m for sucrose of the scutellum SS was found to be 5.8 mM at pH 8 [unpublished results], sucrose concentration would limit the rate of sucrose breakdown only if sucrose was mainly confined to the vacuoles and the cytosolic concentration was kept much below 5.8 mM.

Glucose and UDPG inhibit SS in the breakdown direction [14, 16], and therefore, these compounds were tested on the scutellum SS using saturating levels of sucrose and UDP (Fig. 6). Glucose at concentrations greater than 50 mM caused a 50% inhibition. A maximum inhibition of only 13% was obtained with UDPG at concentrations above 10 mM, whereas UDPG concentrations in the scutellum cells were always less than 4 mM (Fig. 1).

Glucose was much more inhibitory when SS activity in the breakdown direction was measured using UDPG, UDP and UTP at concentrations estimated to be in the cytosol during incubation of the slices in fructose (Fig. 1A). Under these conditions, 100 mM glucose inhibited SS activity 86 to 93% (Fig. 7). The levels of UDPG, UDP and UTP had only a small effect on SS activity, and it is evident that glucose was the principal effector (Fig. 7).

The concentration of glucose in the maize endosperm solution from 3-day-old seedlings was estimated at *ca* 100 mM [17], on the assumption that glucose was evenly distributed in the endosperm water. The concentration at the scutellum surface may be even higher than this, and the scutellum cytosol should contain similar concentrations because the plasmalemma is readily permeable to glucose [15]. Therefore, it is concluded that in the intact seedling, glucose entering the cytosol from the endosperm solution strongly inhibits SS in the breakdown direction and thereby prevents a futile cycle.

The relatively low rates of sucrose breakdown in water incubated slices (Fig. 4) indicate that other inhibitors of SS may be present (although, as discussed above, low cytosolic sucrose concentrations could account for these results). Protein fractions that inhibit SS in the breakdown, but not in the synthesis, direction have been

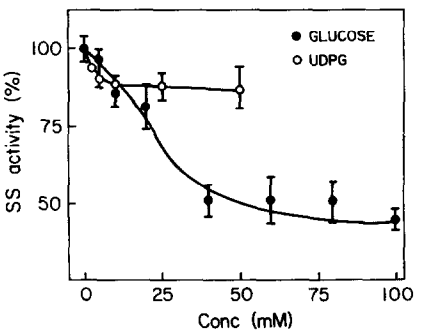

Fig. 6. Effect of glucose and UDPG on the breakdown activity of SS with saturating levels of substrates. Each point is an average of three experiments.

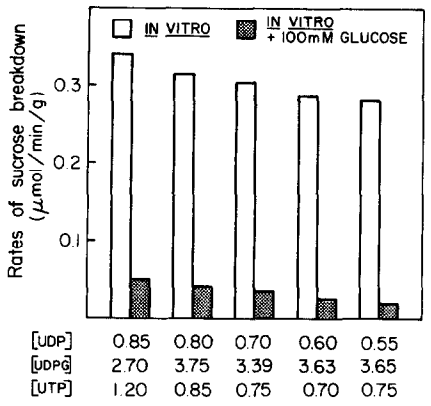

Fig. 7. Effect of 100 mM glucose on the breakdown activity of SS at UDPG, UTP and UDP concentrations found in the slices at each hour of a 5 hr incubation in 100 mM fructose. Each column is an average from two experiments.

isolated from wheat seeds [18]. In the presence of these proteins, the affinity of SS for UDP (but not for sucrose) was changed, and the enzyme exhibited sigmoidal instead of hyperbolic kinetics, which indicate that the proteins are involved in the regulation of SS activity. If similar proteins are present in the maize scutellum, they were either absent or inactivated in the crude extracts used in the experiments of this paper, as indicated by the relatively high SS activity (Fig. 4) and by the hyperbolic kinetics (Fig. 5). Furthermore, *in vivo* breakdown of sucrose in the presence of DNP occurred at a maximum rate [12] that was equal to the maximum *in vitro* rate of SS shown in Fig. 4, which argues against the presence, *in vivo*, of inhibitory proteins.

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 [19].

Breakdown and synthesis of sucrose in scutellum slices. Groups of slices (0.5 mm or less in thickness, 0.5 g) were placed in 50 ml beakers with 10 ml of H_2O or 100 mM fructose and incubated on a gyrotory H_2O bath at 30° for periods up to 5 hr. Net sucrose breakdown or synthesis during these incubation periods were determined as previously described [12, 13].

Extraction and analysis of uridine compounds in scutellum slices. After incubation in H_2O or fructose for periods up to 5 hr, groups of slices (0.5 g) were killed in 5 ml of boiling 50 mM EPPS buffer, pH 8 [20]. After cooling for 30 min, 4 ml of 50 mM $MgSO_4$ were added, the soln was centrifuged (1000 *g*, 10 min) and filtered through Whatman no. 43 paper. Aliquots of 1.9 ml were combined with 0.3 ml EPPS buffer (M, pH 8.2), 0.3 ml ATP (15 mM), 0.3 ml glucose-1-P (15 mM) and 0.2 ml NAD (10 mg/ml). UDPG, UTP, UDP and UMP were determined by measuring the formation of NADH at 340 nm after adding UDPG dehydrogenase (Sigma type VI, 0.1 unit), UDPG pyrophosphorylase (Sigma, 0.5 unit), NDP kinase (Sigma grade IV, 10 units) and NMP kinase (Sigma, 0.1 unit) and following the increase in *A* after addition of each enzyme [21]. When known amounts (*ca* 1.5 × the amounts in fresh slices) of UDPG, UTP, UDP and UMP were added before killing the slices, recovery was 90% or better.

Calculation of internal concns of sucrose and uridine compounds. The slices contained 70% H_2O (350 μl/0.5 g). In calculating internal sucrose concns, it was assumed that 80% (280 μl) of the tissue H_2O was inside the plasmalemma [13] in which sucrose was uniformly distributed. It was assumed that the uridine compounds were uniformly distributed in the cytosol and that the vol. of the cytosol was the same as the glucose space of the slices, which was estimated to be 40 μl/0.5 g or 14.3% of the vol. inside the plasmalemma [19]. To convert the *in vivo* concns of the uridine compounds given in the Figs to μmol/g fr. wt of slices, multiply by 0.08.

Preparation of scutellum extract for SS assay. Extracts were prepared from 10 g of slices as previously described [12]. At pH 8 (the estimated pH of the scutellum cytosol [19]) with the addition of 5 mM NaF, the scutellum extract did not breakdown UDPG, UTP or UDP during incubation at 30° for periods of at least 30 min.

Assay for sucrose synthase. (a) *Synthesis activity.* The reaction mixture contained 100 mM EPPS pH 8; 100 mM fructose; 5 mM NaF; 4 mM EDTA; UDPG, UTP, UDP and glucose at concns shown in the Results section; and scutellum extract in a total vol. of 0.5 ml. To saturate SS, 15 mM UDPG was added (Fig. 3). Reactions were run at 30° for 10 min and stopped by boiling in H_2O for 1.5 min. Control tubes were boiled at zero time. The tubes were centrifuged at 1000 *g* for 10 min. Aliquots were analysed for sucrose [12] and/or UDP. UDP was assayed using pyruvic kinase and lactic dehydrogenase by the procedure described in ref. [21]. (b) *Breakdown activity.* The reaction mixture contained 100 mM EPPS pH 8; 50 mM sucrose; 5 mM NaF; UDPG, UTP, UDP and glucose at concns shown in the Results section; and scutellum extract in a total vol. of 0.5 ml. To saturate SS, 5 mM UDP was added (Fig. 6). Reactions were run at 30° for 10 min and stopped by boiling in H_2O for 1.5 min. Control tubes were boiled at zero time. The tubes were centrifuged at 1000 *g* for 10 min. Aliquots were analysed for UDPG and/or fructose [12]. In the experiments of Fig. 7, SS activity in the breakdown direction was followed in the presence of added glucose by measuring UDP disappearance.

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