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SUCROSE-UDP GLUCOSYLTRANSFERASE OF *ZEA MAYS* ENDOSPERM*

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Key Word Index—*Zea mays*; Gramineae; maize; endosperm; sucrose-UDP glucosyltransferase; sucrose metabolism.

Abstract—The synthetic and degradative activities toward sucrose of maize (*Zea mays* L.) endosperm sucrose-UDP glucosyltransferase preparations behave differently in several respects. Mg^{2+} or Ca^{2+} stimulate the synthetic activity but inhibit the degradative activity. Nucleotides have no effect on the synthetic activity but inhibit the degradative activity. The two activities have different pH optima, and ATP inhibits the degradative activity across the pH range tested. However, both activities exhibit identical patterns of heat inactivation, and various purification procedures employed have failed to separate these two activities. The K_m values at pH 6·5 (degradation) and pH 8 (synthesis) are sucrose, 40 mM; UDP, 0·14 mM; ADP, 1·25 mM; UDPglucose, 1·14 mM; and fructose, 2·08 mM. In the developing endosperm, sucrose-6-P synthetase activity is only *ca* 1% of the synthetic activity of sucrose-UDP glucosyltransferase.

INTRODUCTION

SUCROSE-UDP GLUCOSYLTRANSFERASE (UDPglucose: D-fructose 2-glucosyltransferase, E.C. 2.4.1.13) has been shown to catalyze a reversible reaction[1] as indicated in reaction (1). The equilibrium constant at pH 7·4 has been estimated to be between 1·4 and 5 in favor of sucrose synthesis.[2,3] However, the possibility of a series of reactions in plants, not involving invertase, by which sucrose is degraded and made available for starch synthesis was first suggested by Turner and Turner[4] according to the following scheme:

$$\text{Sucrose} + \text{UDP} \xrightleftharpoons{\text{Sucrose-UDP glucosyltransferase}} \text{UDPglucose} + \text{fructose} \quad (1)$$

$$\text{UDPglucose} + \text{PPi} \xrightleftharpoons{\text{UDPglucose pyrophosphorylase}} \text{glucose-1-P} + \text{UTP} \quad (2)$$

Tsai *et al.*[5] found that a high invertase activity occurs at early stages of endosperm development in maize. At later stages the activity drops sharply to negligible levels while starch synthesis continues. In contrast, there is little sucrose-UDP glucosyltransferase activity at early stages of development, but the activity increases markedly during later stages. These observations suggest that the hydrolysis of sucrose by invertase is only important at early stages of endosperm development. Later, the utilization of sucrose proceeds by way of sucrose-UDP glucosyltransferase and UDPglucose pyrophosphorylase

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as indicated in reactions (1) and (2). The glucose-1-P produced may be utilized in glycolysis or starch biosynthesis in which glucose-1-P is used directly by phosphorylase[6 8] or by conversion to ADPglucose by ADPglucose pyrophosphorylase[9,10] and subsequent incorporation into starch by the starch granule-bound nucleoside diphosphate glucose-starch glucosyltransferase[11,12] and ADPglucose-starch glucosyltransferase.[13] However, DeFekete and Cardini[14] and Murata *et al.*[15] proposed that ADPglucose might also be produced by sucrose-UDP glucosyltransferase, using ADP as a substrate, in addition to the ADPglucose pyrophosphorylase system.

Based on the incorporation of $^{14}CO_2$, Shannon[16] suggested that translocated sucrose is cleaved to glucose and fructose by invertase during entry into maize endosperm. Within the cells, the monosaccharides are rapidly converted to sucrose and thence to starch. If this is correct, the synthesis of sucrose in maize endosperm may be catalyzed by sucrose-6-P synthetase and/or sucrose-UDP glucosyltransferase, although this glucosyltransferase appears to be a sucrose degrading enzyme. Sucrose-6-P synthetase was suggested to function in sucrose synthesis in some plant tissues.[17,18]

The present paper is directed towards investigating, (a) the possibility that the two functions associated with sucrose-UDP glucosyltransferase preparation are attributable to a single enzyme in maize endosperm, and (b) the mechanism by which these two functions are regulated.

RESULTS

Properties of sucrose-UDP glucosyltransferase catalyzing sucrose synthesis and degradation

Sucrose-UDP glucosyltransferase is active over a broad pH range, but the synthetic (sucrose synthesis) and degradative (sucrose degradation) activities exhibit different pH-activity curves. Synthesis is greater at alkaline pH values, with a pH optimum *ca* 8; while degradation is greater at acidic pH, with a pH optimum *ca* 6·5.

At its optimum pH, the synthetic activity is unaffected by 4 mM (final concentration) of ATP, ADP, AMP and only slightly affected by UTP. These nucleotides inhibit sucrose degradation at pH 6·5 to the extent of 80%, UTP; 30%, ATP; 50%, ADP, AMP. Inorganic phosphate has no effect on either reaction (Table 1). ATP inhibits the degradative activity, but not the synthetic, throughout the pH range tested. The inhibition of degradative activity by ATP cannot be relieved by, ADP, AMP or Pi (Table 1).

At a final concentration of 4 mM, Mg^{2+} or Ca^{2+} stimulate the synthesis by 30%, but inhibit degradation. Zn^{2+} strongly inhibits both activities, but K^+ and Na^+ have no effect (Table 2). When the degradative activity was examined at pH 8·0, the effect of various nucleotides and cations was similar to that observed at pH 6·5 (Tables 1 and 2).

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The Michaelis constant (K_m) for different reactants was: for the synthesis (at pH 8), UDPglucose, 1·14 mM and fructose, 2·08 mM, and for the degradation (at pH 6·5), sucrose, 40 mM; UDP, 0·14 mM and ADP 1·25 mM.

TABLE 1. EFFECT OF NUCLEOTIDES ON SUCROSE-UDP GLUCOSYLTRANSFERASE ACTIVITY

Additives (2 μmol)	Activity (A) Sucrose degradation pH 6·5	Sucrose degradation pH 8·0	Sucrose synthesis pH 8·0
None	0·490	0·480	0·300
UTP	0·095	—	0·260
ATP	0·345	0·380	0·300
ADP	0·245	0·250	0·290
AMP	0·230	0·250	0·290
Pi	0·470	—	0·300
ATP + ADP	0·185		
ATP + AMP	0·175		
ATP + Pi	0·340		

Reversibility of the sucrose-UDP glucosyltransferase

Purification procedures failed to separate the synthetic and degradative functions, the activity ratio for the two remaining almost constant (Table 3). Also, both activities exhibited similar patterns for heat inactivation. Protein concentration was critical in studying stability and it was necessary to investigate inactivation at identical protein concentrations. The enzyme preparation was pre-incubated with buffer (pH 6·5 or 8·0) at 55° for varying times and assayed at 30°. At pH 6·5, 1 min of pre-incubation lowers both activities by 50% and 10 min of pre-incubation inactivates both functions completely. At pH 8, pre-incubation for 1 min reduces both activities about 20%; after 10 min of pre-incubation 20% of both activities remained.

TABLE 2. EFFECT OF CATIONS ON SUCROSE-UDP GLUCOSYLTRANSFERASE ACTIVITY

Additives (2 μmol)	Activity (A) Sucrose degradation pH 6·5	Sucrose degradation pH 8·0	Sucrose synthesis pH 8·0
None	0·350	0·330	0·310
$MgSO_4$	0·275	0·200	0·395
$ZnSO_4$	0·0	0·010	0·035
$CaCl_2$	0·315	0·240	0·390
KCl	0·350	0·330	0·310
NaCl	0·355	0·330	0·305

The formation of ADPglucose and UDPglucose by sucrose-UDP glucosyltransferase

The K_m for ADP is about 10 times higher than that for UDP. The formation of ADPglucose or UDPglucose is inhibited by high concentrations of their respective nucleotide (ADP or UDP). Inhibition by ADP occurs at final concentrations higher than

2·5 mM, but inhibition by UDP is only observed at concentrations higher than 10 mM (Fig. 1). The formation of ADPglucose and UDPglucose is sensitive to ATP inhibition.

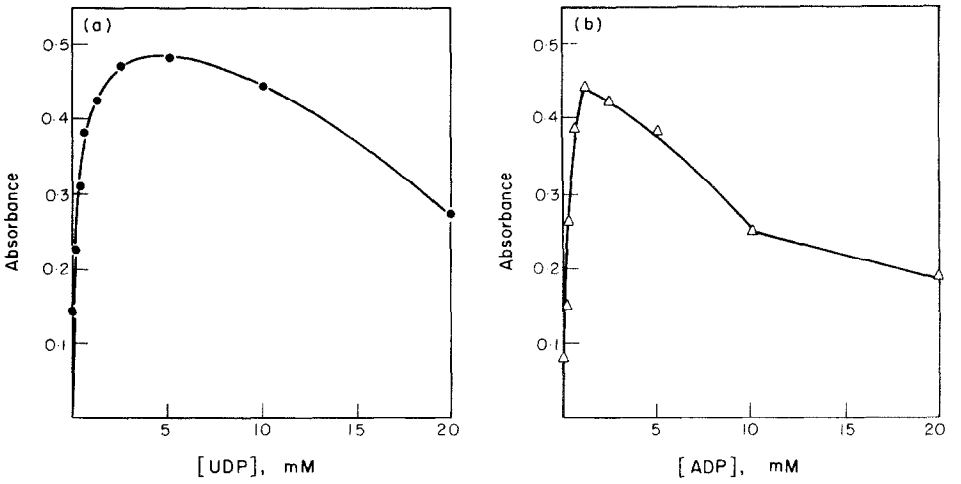

FIG. 1. THE EFFECT OF VARYING UDP (a) AND ADP (b) SUBSTRATE CONCENTRATIONS ON THE ACTIVITY OF SUCROSE-UDP GLUCOSYLTRANSFERASE.

However, the inhibitory effect of ATP is more pronounced on ADPglucose formation than on UDPglucose formation. ATP (4 mM) inhibits UDPglucose formation by 30%, but inhibits ADPglucose formation *ca* 90% (Fig. 2).

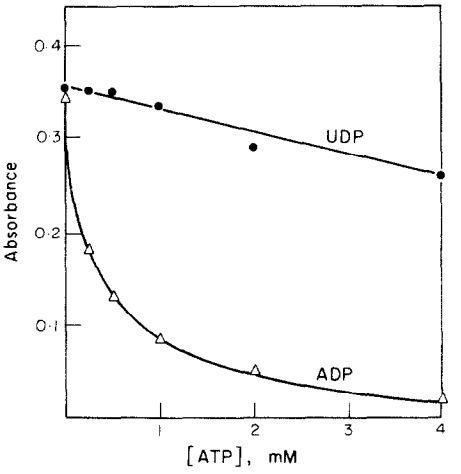

FIG. 2. THE EFFECT OF ATP CONCENTRATION ON SUCROSE-UDP GLUCOSYLTRANSFERASE ACTIVITY UTILIZING ADP OR UDP AS A SUBSTRATE.

The activity of sucrose-UDP glucosyltransferase and sucrose-6-P synthetase in developing endosperm

The determination of sucrose-UDP glucosyltransferase and sucrose-6-P synthetase activities in developing endosperm was generally difficult without preliminary purification because of the presence of invertase and phosphatase in the crude homogenate. However, since the invertase activity was low at 22 days and essentially absent at 28 days

post-pollination,[5] the 28-day stage allowed determination of the activity of both enzymes in the crude homogenate. The sucrose-6-P synthetase activity was low at this stage, i.e. 6 nmol of sucrose formed/endosperm/min as compared to 1050 nmol of sucrose formed/endosperm/min for the synthetic activity of sucrose-UDP glucosyltransferase. At 22 days after pollination, the activities of sucrose-6-P synthetase and sucrose-UDP glucosyltransferase were 5·3 and 1320 nmol of sucrose formed/endosperm/min, respectively. Since NaF did not completely inhibit the activity of phosphatase, which hydrolyzes fructose-6-P to fructose, even the small amount of sucrose formed might have been due to the synthetic activity of sucrose-UDP glucosyltransferase acting on phosphatase-produced fructose.

TABLE 3. SPECIFIC ACTIVITY OF SUCROSE-UDP GLUCOSYLTRANSFERASE THROUGH VARIOUS STEPS OF PURIFICATION

Preparations	Sucrose Synthetic activity Total act. (μmol/min)	Sucrose Synthetic activity Sp. act. (μmol/mg protein/min)	Sucrose Degradative activity Total activity (μmol/min)	Sucrose Degradative activity Sp. act. (μmol/mg protein/min)	Ratio (synthesis/ degradation)
Crude homogenate	144	6·3	110·0	4·7	1·30
40% $(NH_4)_2SO_4$	126	8·4	84·0	5·6	1·50
DEAE-cellulose	62·6	30·0	43·5	20·8	1·44
50% $(NH_4)_2SO_4$	45·0	41·7	32·0	29·6	1·41
G-200	27·0	180·0	19·2	128·0	1·41

DISCUSSION

The synthetic and degradative activities of sucrose-UDP glucosyltransferase preparations behave differently in several respects, e.g. pH optima, cation effects and nucleotide inhibition. However, it has not been possible to separate the two activities by various purification procedures. Also, the two activities exhibit an identical pattern of heat inactivation. The evidence suggests that both synthetic and degradative activities are attributable to the same enzyme. In this respect, the enzyme is similar to that found in potato tuber.[19]

Tsai *et al.*[5] suggested that sucrose-UDP glucosyltransferase is an important enzyme for sucrose degradation in maize endosperm. If so, the inhibition of glucosyltransferase by ATP and UTP suggests a regulatory mechanism for energy metabolism. When levels of ATP are high, the activity of plant phosphofructokinase (PFK) is inhibited[20] leading to an accumulation of fructose-6-P. Sufficiently high levels of fructose-6-P can reverse the ATP inhibition of PFK.[21] The inhibition by ATP and UTP (a product of reaction 2) of sucrose-UDP glucosyltransferase in hydrolyzing sucrose could serve to inhibit the diversion of more glucose-1-P into the glycolytic pathway.

In mammalian systems the activity of animal phosphorylase and PFK is regulated by the ratio of AMP to ATP,[22–24] suggesting that the course of energy metabolism in mammalian systems may be controlled in large part by the AMP/ATP ratio. In plant

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systems, the ATP inhibition of PFK cannot be reversed by AMP.[20,21] The same observations have been made for maize phosphorylase[25] and maize glucosephosphate isomerase.[26] The present studies of sucrose-UDP glucosyltransferase also show that AMP is without effect in relieving the inhibition by ATP.

The activity of sucrose-6-P synthetase is negligible in maize endosperm, amounting to only *ca* 1% of the synthetic activity of sucrose-UDP glucosyltransferase. Even if the total activity detected can be attributed to sucrose-6-P synthetase, this amount of activity is insufficient to account for the rate of sucrose to starch conversion (starch accumulation) in maize endosperm. About 32 mg of starch/endosperm is accumulated within a period of 6 days (22–28 days post-pollination).[5] However, sucrose-6-P synthetase could only produce *ca* 18 mg of sucrose in the same period, which is inadequate to account even for starch synthesis, quite apart from other sucrose utilizing reactions. Thus, it is unlikely that the major portion of sucrose in maize endosperm is synthesized by sucrose-6-P synthetase. Since there is no detectable activity for sucrose phosphorylase in maize endosperm (Tsai, unpublished data), this excludes sucrose synthesis via this enzyme. On the other hand, sucrose may be synthesized by way of sucrose-UDP glucosyltransferase. The ability of sucrose-UDP glucosyltransferase to catalyze sucrose synthesis or degradation could be regulated by pH and the concentrations of UTP, ATP, UDP, ADP, UDPglucose, Mg^{2+} or other unknown factors.

The conversion of sucrose to starch by sucrose-UDP glucosyltransferase utilizing ADP as a substrate seems unlikely. The K_m for ADP is about 10 times higher than for UDP, and the synthesis of ADPglucose is much more sensitive to ADP and ATP inhibition than is UDPglucose formation. Furthermore, the starch-deficient maize mutant, *shrunken*-2, which produces only about 30% of the normal quantity of starch, was found to lack ADPglucose pyrophosphorylase but contain normal levels of sucrose-UDP glucosyltransferase.[10]

EXPERIMENTAL

Plant material. Normal (non-mutant) hybrid, B37 × B14, of maize (*Zea mays* L.) was self-pollinated, harvested at 22 days after pollination (unless otherwise specified), frozen in dry ice in the field and stored at −20°.

Preparation of enzyme. Kernels were cut from the cob, and the embryo and pericarp were removed by dissection. Equal parts (W/V) of endosperms and chilled 10 mM Tris buffer (pH 7·0) were homogenized for 2 min in a blender, strained through 2 layers of cheesecloth, and centrifuged for 20 min at 29000 *g*. From the supernatant, the fraction precipitating between 20 and 40% saturation with $(NH_4)_2SO_4$ was collected at 18800 *g* for 20 min, suspended in cold 10 mM Tris buffer (pH 7·0) and dialyzed against the same buffer for 8 hr at 4°. A sample of the $(NH_4)_2SO_4$ fraction containing approximately 35 mg of protein (measured by the method of Lowry *et al.*[27] with bovine serum albumin as standard) was applied to a DEAE-cellulose column (1 × 20 cm) previously equilibrated with 10 mM Tris buffer at pH 7. The proteins were eluted with a linear, 0–0·5 M NaCl, gradient in 5-ml fractions. Fractions containing sucrose-UDP glucosyltransferase activity were pooled, dialyzed against 10 mM Tris buffer (pH 7·0), and precipitated between 20 and 50% saturation with $(NH_4)_2SO_4$. The pellet was suspended in cold 10 mM Tris buffer, dialyzed against the same buffer for 8 hr at 4° and applied to a Sephadex G200 column (2·2 × 48 cm) pre-equilibrated with 10 mM Tris buffer, pH 7. The proteins were eluted with the same buffer in 5-ml fractions. Specific activity of glucosyltransferase preparations in various stages of purity is presented in Table 3.

Measurement of enzymatic activity. Sucrose synthesis was measured by the method of Roe[28] using a reaction mixture containing 60 μmol of *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 8, 3 μmol of UDPglucose, 2 μmol of fructose and 2 μmol of specific additive. Sucrose degradation was

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followed by the release of reducing sugar[29] in a mixture containing 60 μmol of 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 6·5 or HEPES buffer, pH 8, 20 μmol of sucrose, and 0·5 μmol of UDP or ADP and 2 μmol of specific additive. For both reactions, the quantity of enzyme preparation added and the incubation time at 30° were adjusted so that the final reading was between 0·2 and 0·5 absorption units for a 10 mm light path. No detectable activity for phosphatase or invertase was found in the partially purified enzyme preparations. Kinetic studies were performed under conditions where velocity was linear with respect to both time and enzyme concentration. Sucrose-6-P synthetase activity was determined as described for the synthetic reaction of sucrose-UDP glucosyltransferase except that fructose was replaced by fructose-6-P and 10 μmol of NaF.

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