Chemical Control of Sucrose Conversion to Polysaccharides in Sweet Corn after Harvest

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The conversion of sucrose to polysaccharide in sweet corn depletes the sucrose level during the first day after harvest. Studies of the conversion of sucrose to starch indicate that pyrophosphate was the most effective agent in maintaining the amount of sucrose after harvest. Using adenosine diphosphate–glucose pyrophosphorylase extracted from sweet corn, an effective inhibition of adenosine diphosphate–glucose synthesis by pyrophosphate was demonstrated *in vitro*. The effect of pyrophosphate treatment is interpreted as an end product inhibition of nucleotide diphosphate–glucose synthesis.

The rapid conversion of soluble sugar to polysaccharides after harvest has long been a problem in maintaining quality produce for marketing. Quantitative data available in the literature show a depletion of 40–60% of the sucrose originally present in sweet corn after 24 hr storage at 25° C (Appleman and Arthur, 1919; Culpepper and Magoon, 1924; Doty *et al.*, 1945). The rapid deterioration in quality is only partially reduced by storage at low temperature; refrigerated samples of sweet corn, supplied to our laboratory by commercial sources, were below 50% of the amount of sucrose expected at harvest time. The chemical reactions involved in starch biosynthesis in corn have been extensively studied using different maize endosperm mutants characterized by variation in their starch–sucrose levels (Black *et al.*, 1966; Creech, 1965; De Fekete and Cardini, 1964; Dickinson and Preiss, 1969; Shannon *et al.*, 1970; Tsai and Nelson, 1966; Tsai *et al.*, 1970).

Starch biosynthesis in corn endosperm and in photosynthetic tissues is regulated by nucleotide diphosphate–glucose pyrophosphorylase (NDP–glucose pyrophosphorylase) activity. The enzyme is activated by glycerate 3-P (PGA) and inhibited by inorganic phosphate (Dickinson and Preiss, 1969). If starch accumulation in endosperm is regulated by nucleotide diphosphate–glucose (NDP–glucose) synthesis, this reaction might be a vulnerable point of controlling starch synthesis. In analogy with other reactions involving nucleotides which release pyrophosphate (El-Badry and Bassham, 1970; Kornberg, 1962) the pyrophosphate (PPi) level by end product inhibition could be a controlling factor in NDP–glucose synthesis in corn endosperm. End product inhibition by PPi might be expected only if pyrophosphatase (PPiase) is not active in endosperm cells. However, it is known that photosynthetic tissues contain considerable PPiase activity (Simmons and Butler, 1969). Because various hormones regulate enzymatic activities, the use of plant hormones was also considered in the control of sucrose conversion to starch. Varner (1964) has shown that GA is effective in controlling the activity of α-amylase in barley endosperm. Also, Cherry (1968) demonstrated that GA is effective in regulating the synthesis of invertase in storage tissues of sugar beet.

The purpose of this study was to investigate the possibilities of biochemical control of sugar conversion to polysaccharides in sweet corn.

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MATERIAL AND METHODS

Reagents. Protamine sulfate grade 1, ^{14}C-D-Glucose-1-P, ATP, dATP, CTP, GTP, UTP, and tetrasodium pyrophosphate were obtained from Sigma Chemical Co. ^{32}P-inorganic pyrophosphate was purchased from Amersham/Searle. DEAE-cellulose paper DE81 was obtained from Whatman, and invertase (yeast) grade B was purchased from Calbiochem Co.

Experimental Conditions. Except for the ears obtained from commercial sources, the corn plants were hand pollinated to insure uniform maturity of the ear. All samples consisted of two rows of kernels taken from the center of the cob. Two border rows of kernels were always left on each side of the sample area. After kernels were removed the husk was replaced to guard against desiccation. For sugar analysis 4-g samples of kernels were immediately dropped in boiling 80% ethanol and then stored under refrigeration.

Sugar Extraction. After homogenization in a Omnimixer blender for 3 min, the sugars were extracted for 2 hr with 80% ethanol in a Soxhlet extractor using a fiber glass thimble. Reducing sugars were determined by Nelson's (1944) method. Sucrose was hydrolyzed to reducing sugar by invertase followed by Nelson's determination. The difference between total reducing sugar after invertase treatment and reducing sugar before enzyme hydrolysis was used as a measure of sucrose content. The dry weight (D.W.) was determined by drying the sample in an oven at 60° C for 38 hr. Protein was determined by the method of Lowry *et al.* (1951).

Introduction of Treatment. The chemicals were introduced with a hypodermic needle (containing a wire to prevent clogging) in the central parenchyma tissue of the ear. A solution of 5 ml was introduced twice at intervals of 8 hr. A rubber tube containing 10 ml of the same solution was attached to the ear at the bottom to ensure an adequate supply of the chemical. With detached ears the treatment was applied 24 hr before introducing the ears to room temperature (0 time).

Assay for Adenosine Diphosphate Glucose Pyrophosphorylase. Pyrophosphorolysis activity and synthesis of ADP–glucose was assayed by the methods of Dickinson and Preiss (1969), except that the Norit A was filtered onto glass fiber filters (GF/A Whatman, 2.1 cm) and counted in a liquid scintillation counter. The presence of Norit A in the toluene scintillation system reduced counting efficiencies by 25%. In assaying the enzyme in the direction of ADP–glucose synthesis, the alkaline phosphatase hydrolysate was adsorbed

Table I. Translocation of Sucrose[a] Injected into the Cob to the Kernels

Time, hr	Sucrose uptake: Unlabeled mg/g D.W.	Sucrose uptake: Labeled[b] ^{14}C DPM/g D.W.
0	77.4	0
1	74.6	65,720
2	85.9	176,520
6	109.5	439,200
12	126.0	761,996

[a] The sucrose concentration introduced into the ears was 0.3*M*. [b] Radioactivity from the ethanol extraction was expressed as disintegration per minute (DPM).

on a stack of 6 DEAE-cellulose paper discs and then washed, dried, and counted.

Assay of Pyrophosphatase (PPiase). The activity of this enzyme was measured according to the method of Simmons and Butler (1969).

RESULTS AND DISCUSSION

Changes in Reducing and Nonreducing Sugar During Storage. Changes in the content of soluble sugar of corn ears grown and handled under different conditions are shown in Figure 1. The decrease in sucrose content is most rapid in the first 24 hr of storage at room temperature, as reflected by a loss of as much as 50% of the sucrose. In general, corn having a large initial level of sucrose exhibits a much larger percentage of sucrose lost than ears containing initially low sucrose contents. The amount of reducing sugar in the three sources of corn varies much less following an initial decline of about 20%. During the first 12 hr of storage the reducing sugars remain virtually unchanged.

Translocation and Distribution of Compounds in Detached Ears. The use of detached ears as an experimental system required some information about the translocation and penetration of chemical treatments into the kernels. By injecting labeled and unlabeled sucrose into the cob, it was shown that translocation into the kernel was quite rapid (Table I). Radioactivity was detected in the kernels within 1 hr after injection.

The Influence of Different Treatments on Sucrose Level. The plant hormones [zeatin, gibberellic acid (GA_3)] and Pi had no effect on the conversion of sucrose to starch during the 24 hr of storage at room temperature (Figure 2). Only the PPi treatment maintained the sucrose level. The finding that orthophosphate has no effect on the conversion of sucrose to starch is a preliminary indication that the pyrophosphate ester remains intact. However, the results do not eliminate the possibility that PPiase might decrease the efficiency of PPi. This point was further checked by experiments using different concentrations of PPi; treatments above 1 m*M* had no further effect on the sucrose level (Table II). The observation that PPi may be acting as a chelating

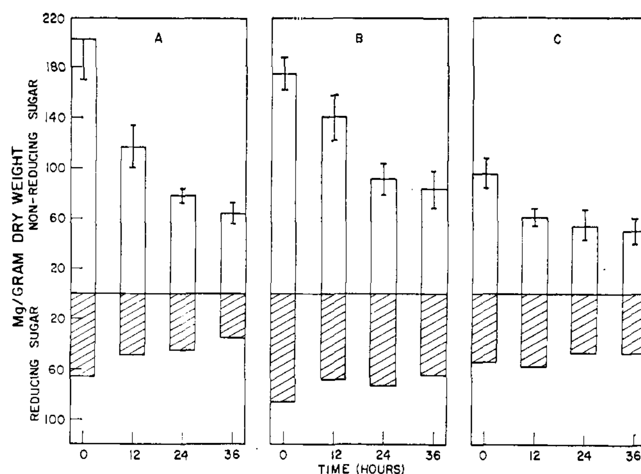

Figure 1. Changes in reducing and nonreducing sugar in sweet corn during postharvest storage at 25° C. A. Fresh ears from the field. B. Fresh ears from greenhouse. C. Stored ears in refrigeration from commercial source. Vertical bar = standard deviation (S.D.)

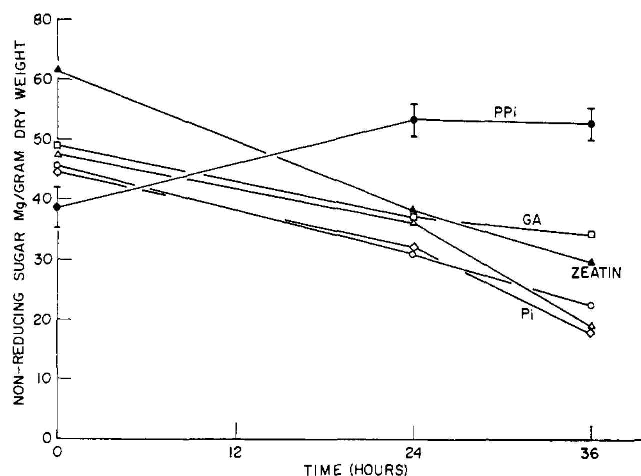

Figure 2. Effect of PPi, Pi, GA_3 and zeatin on sucrose level during storage period of 36 hr at 25° C. Vertical bar = S.D. ● = PPi. ◇ = Pi. □ = GA_3. ▲ = zeatin. ○ = control H_2O. △ = control mannitol

Table II. Influence of Different Concentrations of PPi and EDTA on Sucrose and Reducing Sugar Level in Detached Sweet Corn Ears during 24 Hr at 25° C

	Concentration, m*M*	Sucrose mg/g D.W. Initial	Sucrose mg/g D.W. After 24 hr	Sucrose % Decrease	Reducing sugar mg/g D.W. Initial	Reducing sugar mg/g D.W. After 24 hr	Reducing sugar % Decrease
Control		66.8	31.6	53	56.3	56.7	
Control H_2O		53.3	29.3	46	47.6	40.5	15
Control mannitol	10	75.5	37.4	51	57.7	51.6	11
PPi	1	60.0	53.4	11	41.5	38.7	7
PPi	5	63.0	59.4	6	54.3	42.5	22
PPi	10	77.6	57.3	27	52.0	50.0	4
EDTA	1	55.3	29.2	48	43.7	42.1	4
EDTA	5	61.5	27.5	66	44.4	37.9	15
EDTA	10	55.8	30.9	45	47.6	41.9	12

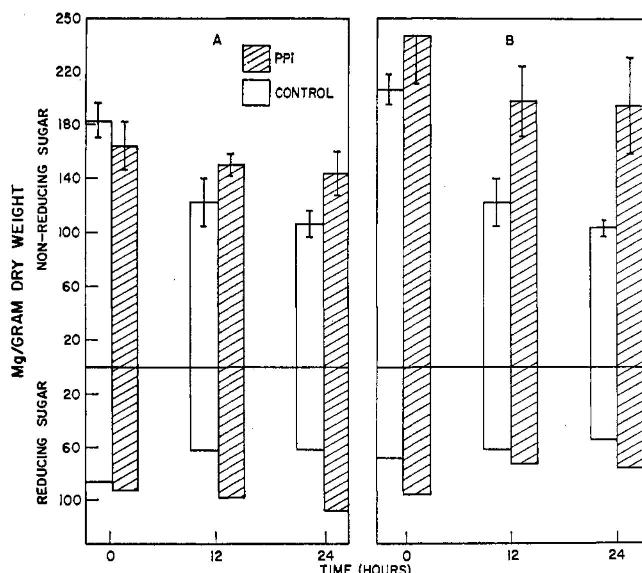

Figure 3. Effect of PPi treatment of attached ears, changes in sugars during postharvest storage at 25° C. A. Ears from greenhouse. B. Ears from the field

Table III. Purification of Sweet Corn ADP–Glucose Pyrophosphorylase

Fraction	Volume, ml	Protein, mg	Total units[a]	Specific activity
Crude extract	250	1880	720	0.38
Protamine sulfate	300	1680	782	0.52
Ammonium sulfate	40	588	676	1.14
DEAE-cellulose	50	6	65	11.60

[a] One unit equals 1 μmol of ATP formed in the pyrophosphorolysis assay in 10 min.

Table IV. Requirements for ADP–Glucose Synthesis

	% of Activity
Complete system	100[a]
Complete system − 3 PGA	89.0
Complete system − $MgCl_2$	6.1
Complete system − enzyme	2.7
Complete system − ATP	3.1
Complete system − glucose-1-P	1.3
With dATP[b]	1.7
With GTP	4.2
With UTP	7.6
With CTP	2.1
With $MgCl_2$, 5.0 m*M*[c]	92.0
With $MgCl_2$, 2.5 m*M*	97.0

[a] 100% = 9.7 mμmol of ADP-glucose formed under standard assay conditions. [16 μmol HEPES buffer, *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid, pH 7.7; 0.2 μmol of ATP, 50 μg of bovine serum albumin; 2 μmol of $MgCl_2$; 0.1 μmol of ^{14}C-glucose-1-P (2.5 mCi/mmol); 2 μmol of 3 PGA, and enzyme to a final volume of 0.2 ml]. [b] The ATP was substituted by the other nucleotides in the standard assay system. [c] The complete system contains 10 m*M* $MgCl_2$.

Table V. The Inhibitory Effect of PPi on ADP–Glucose Produced in the Presence of High and Low $MgCl_2$ Concentration

Concentration, PPi, m*M*	mμmol of ADP–Glucose produced	
	$MgCl_2$, 10 m*M*	$MgCl_2$, 20 m*M*
0	8.10	7.24
0.5	2.64	2.18
1.0	1.09	0.79
1.5	0.14	0.1
2.0	0.08	0.02

agent led to the studies of its effect with equimolar concentrations of ethylenediamine tetraacetic acid (EDTA). The results of this experiment are shown in Table II. No effect of EDTA on sucrose was observed.

Influence of Pyrophosphate Treatment on Ears Attached to the Plant. The effect of PPi on ears attached to the plant was investigated in the greenhouse and in the field. PPi (10 m*M*) was applied by injection into the ears 48 hr before harvest (Figure 3). Ears treated on plants with PPi maintained their sucrose level for 24 hr after harvest. Subsequently there was a rapid drop in the sucrose content during storage similar to that for untreated detached ears. The data presented in Figure 3 show a slight increase in reducing sugar. However, from many other experiments, this observation was not always noted.

Effect of Pyrophosphate on Adenosine Diphosphate Glucose Pyrophosphorylase Activity. Based on the hypothesis that PPi effects starch synthesis *via* ADP–glucose synthesis, we extracted the ADP–glucose pyrophosphorylase from sweet corn and assayed its activity. The results of partial purification of the enzyme are shown in Table III. The enzyme assays were carried out within the linear range of enzyme activity with respect to time and protein concentration. The specificity of the enzyme and its requirement are shown in Table IV. Using increasing concentrations of PPi in the ADP–glucose synthesis assay, an effective inhibition range was noted (Table V). Concentrations of PPi above 1.75 m*M* gave a visible turbidity; presumably a Mg-pyrophosphate complex is formed. Addition of Mg^{2+} at levels higher than 10 m*M* did not prevent inhibition of the ADP-glucose synthesis by pyrophosphate. In our experimental conditions used to assay the enzyme, the ratio of $MgCl_2$ to $Na_4P_2O_7$ was kept at 4, where no turbidity in the incubation mixture was noted.

Pyrophosphate has been shown to inhibit enzymatic reactions in several systems, including protein synthesis, fatty acid activation, and nucleic acid synthesis. The inhibition is avoided, *in vivo*, by the hydrolytic breakage of PPi ester linkage by PPiase (Hatch *et al.*, 1969; Kornberg, 1962). According to Simmons and Butler (1969), the activity of PPiase is very low in corn endosperm in comparison with that of green tissues. Our experiments sustain those findings. The PPiase activity in sweet corn endosperm was 0.7 units/g F.W., and is approximately the same activity found in dent corn. We further showed that orthophosphate treatment has no effect on sucrose content during the rapid conversion of sucrose to starch.

The cessation of sucrose–starch conversion by PPi can be interpreted as an end product inhibition of ADP–glucose synthesis. The sugar nucleotides are suggested to be the major precursor for starch synthesis in corn (De Fekete and Cardini, 1964; Nordin and Kirkwood, 1965). Therefore, a depletion of sugar nucleotides will effect the rate of starch synthesis. On the basis of the present finding it seems possible to regulate the conversion of sucrose to polysaccharides by treatment of ears with pyrophosphate.

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