SOLUBLE ENZYMES RELATED TO STARCH SYNTHESIS

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A glucan synthetase[§], bound to the starch grains, has been demonstrated in plants (Leloir et al., 1961; Recondo and Leloir, 1961; Frydman, 1963; Fekete and Cardini, 1964; Murata and Akazawa, 1964). This enzyme catalyzes the glucose transfer from adenosine diphosphate D-glucose (ADPG), deADPG, and uridine diphosphate D-glucose (UDPG) to maltooligosaccharides and starch. All attempts made to separate the enzyme from the granules or to demonstrate its presence in a soluble state have failed.

The presence of another glucan synthetase which transfers glucose residues from ADPG and deADPG to maltooligosaccharides, amylopectin, plant and animal glycogen was found to exist in the sweet corn in a soluble form. Amylose, soluble starch and starch granules were completely ineffective as primers (Frydman and Cardini, 1964).

In an attempt to elucidate the relation between these glucan synthetases, their presence in plants which contain starch as sole reserve polysaccharide, was assayed. Phytoglycogen, amylopectin and corn starch granules (prepared according to Schoch, 1957) were tested as glucosyl acceptors. Tobacco leaves and potato tubers were used as sources for the enzymatic preparations.

Glucan synthetase from tobacco leaves.-Chloroplasts were prepared in an anhydrous medium according to Stocking (1959) and suspended in 0.25 M glycine buffer, pH 8.6-0.025 M versene. For the preparation of an aqueous leaf extract, fresh leaves were ground in a chilled mortar with 0.02 M Tris buffer, pH 7.5-0.002 M mercaptoethanol, passed through cheese cloth and centrifuged for 15 min at 1,000 x g. The supernatant fluid was cen-

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[§]Systematic name: ADPG:α-1,4-glucan α-4-glucosyltransferase.

trifuged again at 18,000 x g for 15 min. The dark green liquid, separated from the precipitate, was used as enzyme source. It contained about 0.5 mg/ml of protein. Precipitation with ammonium sulfate did not inhibit the enzymic activity. Some of the results obtained with this extract and the chloroplast preparation can be seen in Table I.

TABLE I

The reaction mixture contained (in μmoles): NaF, 1.0; glycine buffer, pH 8.6, 3.3; EDTA, 0.082; ADPG-C^{14}, 0.012 (3,900 counts/min) or C^{14}-glucose-1-P, 0.013 (18,000 counts/min) and glucose-1-P, 0.22; primers as indicated and 20 μl of enzyme preparations. Final volume, 80 μl. Incubation time 60 min at 37°. The radioactivity was measured as previously described (Frydman and Cardini, 1964).

Substrates	Primers	Incorporation into the precipitate	
	mg	counts/min	%
Aqueous preparation			
Control[a]		10	
Control[b]		11	
ADPG-C^{14}	amylopectin, 0.5	528	13
ADPG-C^{14} + glucose-1-P	"	480	12
C^{14}-glucose-1-P	"	120	1.5
ADPG-C^{14}	phytoglycogen, 0.5	820	21
ADPG-C^{14} + glucose-1-P	"	800	21
C^{14}-glucose-1-P	"	30	
ADPG-C^{14}	heated starch granules, 3.0	378	10
ADPG-C^{14}	whole starch granules, 3.0	8[c]	
Chloroplast preparation			
Control[a]		2	
ADPG-C^{14}	heated starch granules, 1.0	501	12
ADPG-C^{14} + glucose-1-P	"	410	10
C^{14}-glucose-1-P	"	502	6.3

[a] Primer added at the end of the incubation.

[b] ADPG-C^{14} added at the end of the incubation.

[c] A control in which the enzyme was added at the end of the incubation (64 counts/min) was substracted.

Using ADPG-C^{14} as substrate, a 10%, 13% and 21% incorporation was obtained into heated starch granules, amylopectin and glycogen respectively. The radioactivity incorporated was recovered in all cases as maltose after the action of β-amylase. No incorporation was detected when

the primers were omitted, or when whole starch grains were used. Addition of unlabeled glucose-1-P did not modify the incorporation. UDPG-C^{14} was inactive as glucosyl donor.

The aqueous preparation contained a slight phosphorylase activity (1.5% incorporation). A higher phosphorylase activity was found in the chloroplast preparation (6.3% or higher).

In addition to the polysaccharides mentioned above, maltotriose was found to be an acceptor of glucosyl residues. The radioactive products were identified as maltotetraose and maltopentaose on paper chromatography.

Glucan synthetase from potato tubers.-Potato tubers were peeled, cut in slices, left 5 min in 1% $Na_2S_2O_4$ solution, washed, grated, passed through cheese cloth and centrifuged at 1,000 x g for 15 min. The supernatant fluid was centrifuged at 18,000 x g for 10 min, the precipitate was discarded and the liquid, dialyzed overnight against 0.01 M Tris buffer, pH 7.5-0.01 M mercaptoethanol, was used as enzyme. The extract contained 3.2 mg/ml of protein. Some of the results obtained can be seen in Table II.

ADPG functioned as the best glucosyl donor and the whole starch granules were the only effective glucosyl acceptors. Incorporations ranging between 5% and 20% were obtained. The 1.6% incorporation detected when the enzyme extract was omitted, was probably due to a residual starch synthetase activity. When the starch granules were pretreated with hot methanol, those blanks disappeared, without modifying the primer efficiency. The radioactivity incorporated into the starch granules was recovered as maltose after the action of β-amylase. Addition of unlabeled glucose-1-P did not modify the incorporation from ADPG. Amylopectin, glycogen and heated starch granules did not act as glucosyl acceptors, even when the incubations were made for shorter times, to prevent amylase degradation. A 0.6% incorporation was detected from UDPG-C^{14}, but the same was completely inhibited by the addition of unlabeled ADPG. GDP-C^{14}-glucose and TDP-C^{14}-glucose were ineffective as glucosyl donors.

The activity curves as a function of enzyme concentration and time are shown in Fig. 1. The type of curves obtained could be explained by the low capacity of the primer.

Discussion.-The specificity toward the glucosyl donor and acceptor of the tobacco leaf preparation was similar to that of the sweet corn phytoglycogen synthetase. The fact that this enzyme was found in leaves which contain starch as the sole reserve polysaccharide, would suggest that it is in some way related to starch synthesis.

TABLE II

The reaction mixture contained (in μmoles): NaF, 1.0; Tris buffer, pH 8.6, 1.0; substrates as in Table I, and 60 ul of the potato enzyme preparation. This mixture was incubated with stirring in a volume of 120 μl at 37° for 60 min. The incorporation of radioactivity into the polysaccharides was measured as described previously (Leloir et al., 1961; Frydman and Cardini, 1964).

Substrates	Primers	Incorporation into the ethanol precipitate	
	mg	counts/min	%
Control[a]		5	
Control[b]	amylopectin, 0.5	2	
C^{14}-glucose-1-P	"	280	3.5
ADPG-C^{14}	"	3	
Control[b]	phytoglycogen, 0.5	3	
C^{14}-glucose-1-P	"	2	
ADPG-C^{14}	"	5	
Control[b]	starch granules, 3.0	64	1.6
C^{14}-glucose-1-P	"	3	
ADPG-C^{14}	"	464	12
ADPG-C^{14} + glucose-1-P	"	467	12

[a]Primer added at the end of the incubation.

[b]Enzyme added at the end of the incubation.

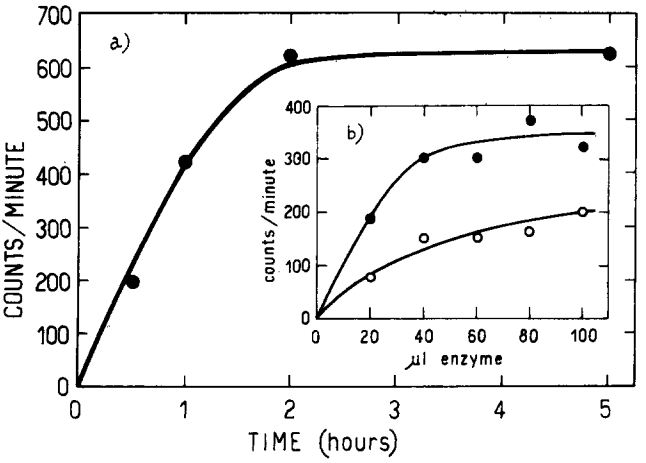

Fig. 1. a) Rate of incorporation of glucosyl residues from ADPG-C^{14} into the product, assayed as described in Table II, except that 5 mg of starch granules were used.
b) Effect of increasing enzyme concentration, assayed as described in Table II, except that two primer concentrations were used (●, 3 mg; ○, 2 mg).

The potato enzyme seems to be similar to the starch synthetase bound to the granules, and may be the soluble form of this enzyme. It is possible that the previous inability to detect the starch synthetase in a soluble state was due to the specific primer requirement rather than to the solubility of the enzyme.

The possible relation between these enzymes is being investigated.

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