BIOSYNTHESIS OF PHYTOGLYCOGEN FROM ADENOSINE DIPHOSPHATE D-GLUCOSE IN SWEET CORN

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The presence of an enzyme which catalyzes glucose transfer from UDP-D-glucose, ADP-D-glucose and deADP-glucose to starch and oligosaccharides was demonstrated in plants (Leloir, Fekete and Cardini; Recondo and Leloir, 1961; Frydman, 1963). The enzyme is bound to the starch granule and all attempts made in order to separate it or to demonstrate its presence in a soluble state, have so far failed.

In the kernels of sweet corn there exists, besides the starch granules, a soluble polysaccharide related to amylopectin, phytoglycogen (Huelsen, 1954; Whelan, 1955). On the basis of structural similarity with animal glycogen, the same biosynthetic mechanism might be suspected to be operative (Leloir and Cardini, 1957).

The presence in the endosperm of sweet corn of an UDP-(ADP)-glucose-fructose glucosyl transferase (Cardini and Recondo, 1962); an ADP-D-glucose pyrophosphorylase (Espada, 1962), and a system active for the synthesis of starch starting from sucrose (Fekete and Cardini, 1963) have already been demonstrated.

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Using a variety of sweet corn very rich in glycogen, we have been able to demonstrate the existence of an enzyme which transfers glucose from ADP-D-glucose to phytoglycogen. In varieties poor in glycogen, the results were inconclusive or negative.

Kernels collected in the succulent stage of growth (milky stage) were ground slightly in a chilled mortar, passed through cheese-cloth and the resulting milky liquid was centrifuged at 1,000 x g for 15 minutes. All operations were carried out at 0° C. The supernatant fluid was centrifuged at 18,000 x g for 30 minutes. The opalescent liquid was then carefully separated from the precipitate and the oily layer, and was centrifuged in a Spinco preparative ultracentrifuge at 100,000 x g for 2 hours. An abundant whitish traslucid precipitate, covered by a yellow jelly and consisting mainly of glycogen, was obtained. The clear supernatant liquid was carefully separated and dialyzed for 3 hours against distilled water at 0° C. It contained about 15 mg/ml of protein and 0.1 mg/ml of glycogen and was used as the enzyme preparation. A 24-hour dialysis and even precipitation with ammonium sulfate did not alter the activity of the enzyme.

Some of the results obtained with this extract can be seen in Table I. Using ADP-C^{14}-D-glucose as substrate a 24% incorporation into glycogen was obtained. A 0.5% incorporation was detected when glycogen was not added. Animal glycogen was as good a primer as phytoglycogen. The activity curve as a function of the amount of primer can be seen in Fig. 1. Nearly all the radioactivity incorporated into glycogen was recovered as maltose after the action of β-amylase. Addition of unlabeled glucose-1-P did not modify the incorporation. No radioactivity was incorporated from UDP-C^{14}-glucose or from C^{14}-glucose-1-P alone or with 5'-AMP (Frederick, 1963). Glucose-6-P does not seem to modify the activity of the enzyme, at variance from animal tissues and yeast (Leloir and Goldem-

TABLE I

The reaction mixture contained: 1 μmole of FNa; 0.5 μmole of Tris buffer pH 7.6; 1 mg of phytoglycogen prepared according to Whelan (1955); substrates as indicated, and 20 μl of enzyme preparation. This mixture was incubated in a volume of 70 μl for 30 minutes at 37° C. The reaction mixture was heated for 20 minutes with 0.6 ml of KOH 33%. Glycogen was precipitated by addition of 1 ml of ethanol, centrifuged, redissolved in 0.2 ml of water and reprecipitated with 1 ml of ethanol. This procedure was repeated 3 times. The glycogen was then plated on aluminum disks and counted with a gas flow counter (Tracerlab, Inc.).

Substrates	Incorporation into glycogen	
(μmoles)	(counts/minute)	(%)
Control[a]	2	
Control[b]	10	
ADP-C^{14}-D-glucose, 0.22 (1,300 counts/minute)	320	24
UDP-C^{14}-D-glucose, 0.16 (18,000 counts/minute)	18	
C^{14}-Glucose-1-P, 0.16 (15,000 counts/minute)	40	
ADP-C^{14}-D-glucose, 0.22 (1,300 counts/minute) + glucose-1-P, 0.2	303	22
ADP-C^{14}-D-glucose, 0.22 (1,300 counts/minute) + glucose-6-P, 0.2	340	25
ADP, 0.16 + sucrose-C^{14}, 0.1 (45,000 counts/minute)	430	1
UDP, 0.16 + sucrose-C^{14}, 0.1 (45,000 counts/minute)	15	

[a]Reaction mixture contains ADP-C^{14}-D-glucose. Glycogen added at the end of the incubation.

[b]Reaction mixture contains glycogen. ADP-C^{14}-D-glucose added at the end of the incubation.

berg, 1960; Algranati and Cabib, 1962). ADP and sucrose-C^{14} could replace ADP-D-glucose by way of the ADP-glucose-fructose glucosyl transferase (Cardini and Recondo, 1962). The dilu-

tion with the unlabeled sucrose present in the extract could explain the low incorporation.

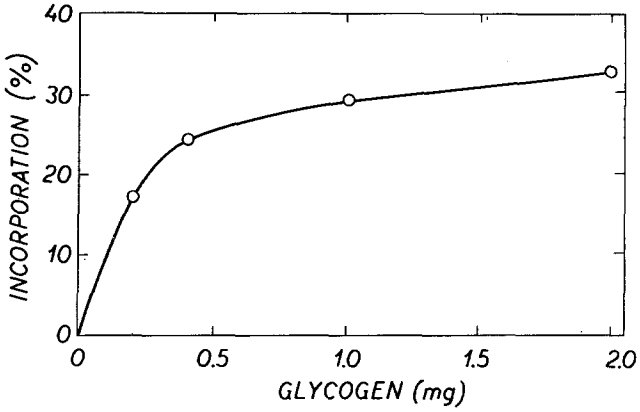

Fig. 1. Effect of glycogen concentration; assayed as described in Table I.

In another series of experiments it was demonstrated that C^{14}-glucose-1-P could act as substrate, but in the presence of ATP and Mg^{++} through ADP-glucose pyrophosphorylase (Espada, 1962).

In this enzymatic extract there is also an active system which transforms amylose and amylopectin into glycogen.

It is interesting to point out the different specificity for the glucosyl donor in the synthesis of animal glycogen, starch and phytoglycogen. While UDP-glucose and ADP-glucose serve as glucosyl donors for the first two polysaccharides, ADP-glucose is the one active on the synthesis of phytoglycogen. This would suggest that starch and phytoglycogen in sweet corn are synthesized by two different enzymes. ADP-glucose has been isolated from this variety of corn (Recondo, Dankert and Leloir, 1963).

Further studies on this enzyme are being carried out.

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