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A NEW ADP (UDP) - GLUCOSE GLUCOSYLTRANSFERASE ACTIVITY IN DISRUPTED STARCH GRAINS

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SUMMARY - Disrupted potato starch granules obtained in the presence of 8 M urea were shown to increase [^{14}C] glucose incorporation from labeled ADP-glucose or UDP-glucose into starch, as compared to intact grains. Labeled glucose or maltose units were found to be incorporated through a linkage that produced cyclic phosphate esters upon mild alkaline treatment and was sensitive to hydrolysis at pH 2.0. Both properties of this linkage strongly resembled those of the pyrophosphate bond of ADP-glucose or UDP-glucose.

In previous work (1-3), an enzymatic system has been described in proplastids of potato tuber, capable of forming α-1,4 glucosidic chains from UDP-[^{14}C]glucose or ADP-[^{14}C]glucose and an endogenous proteic acceptor, that yielded a proteoglucan. The proteic acceptor, which would act as primer, could be the enzyme itself, as it is the case for potato phosphorylase (4). This proteoglucan would be assumed as the initiator of starch biosynthesis. If this mechanism is actually operating in the formation of the grain, the possibility exists that starch synthetase bound to the grain (5) remains covalently linked to amylose or amylopectin. In order to test this hypothesis, it becomes necessary to destroy the structure of the starch granule to allow the isolation of its constituent polysaccharides. Two methods were employed to accomplish that: a) grinding of granules or b) treatment with 8 M urea (6-9). An increase in the incorporation of radioactivity from ADP-[^{14}C]glucose was already ob-

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served in the granules incubated under the above conditions, with respect to intact starch granules (6, 7). Concerning to UDP-[^{14}C]glucose as glucosyl donor, contradictory results were reported (6, 9). In none of these cases the resulting labeled compounds have been studied, in the assumption that they were indistinguishable from those obtained with intact grains.

In this paper, analysis of the labeled material obtained in the presence of 8 M urea is presented. The increase of [^{14}C]glucose incorporation from ADP-[^{14}C]glucose into starch was confirmed (6, 9). Data from other authors (10), regarding stimulation from UDP-[^{14}C]glucose, were also verified, in disagreement with that previously reported (6, 9). It seems very likely that the observed discrepancies were due to the different techniques employed in each case for the disruption of granules. Searching for the fate of the incorporated glucose, it was found that [^{14}C]glucose was not used up for the elongation of amylose or amylopectin chains, as in intact grains; but it was found as glucose or maltose units probably linked to protein through a labile bond which resembles the pyrophosphate linkage.

MATERIALS AND METHODS

ADP-[^{14}C]glucose and UDP-[^{14}C]glucose were synthesized according to Thomas et al. (11). Urea was recrystallized once from 70% methanol. Freshly prepared solutions were used throughout this work. The preparation of starch grains as well as the methods employed were already described (5).

Assay Mixture: 5 mg of potato starch grains were mixed with 50 µl of water or 8 M urea, as indicated in each case. In order to get an homogenous mixture, 1 µl of the radioactive glucosyl donor was first added to the starch and then, the urea solution. The reaction mixture was vigorously stirred in a Vortex until gelification occurred. Incubations were carried out at 37ºC for 60 min. The reaction was stopped by the addition of 1 ml of 70% methanol-1% KCl. The tubes were centrifuged, the precipi-

tate was washed three times with 1 ml of the same solution and twice with hot methanol in order to eliminate traces of [^{14}C] sugar nucleotides that might contaminate the precipitate.

Proteolytic Digestions: The radioactive insoluble material dissolved in 8 M urea was digested with pronase, subtilisin and chaotropase as described (3, 12). Final concentration of urea during proteolysis was 1.6 M. Controls were run to preclude any interference of urea with proteolytic enzymes.

Paper chromatography was done on Whatman Nº 1 paper using the following solvent systems: 3 M NH_3 in 80% (v/v) ethanol (13) (solvent A); n-butanol-pyridine-water (6/4/3) (solvent B); ethanol-1 M ammonium acetate brought to pH 7.4 with NH_4OH (75/30) (solvent C); and n-butanol-pyridine-water (4/3/4) (14) (solvent D). With solvent A, the ascending technique was followed.

Paper electrophoresis was performed with Whatman Nº 1 paper at 1000 V (20 V/cm) for 2.5 h in 1.2 mM pyridinium acetate, pH 6.5 (buffer A).

RESULTS

Table I shows [^{14}C]glucose incorporation in intact starch grains or in disrupted grains in the presence of 8 M urea. It can be seen that the treatment with urea led to an increase in the incorporation of radioactivity. When the radioactive intact grain was submitted to α-amylase digestion, it yielded glucose, maltose and a low proportion of higher malto-oligosaccharides, as already described (15). On the other hand, paper chromatography in solvents B or D of the α-amylase digest obtained from labeled disrupted granules, showed most of the radioactivity at the origin; only a small peak of [^{14}C] glucose was detected on the chromatogram. A hint on the anionic nature of this labeled material was gathered from paper electrophoresis in buffer A and paper chromatography in solvent C (it migrated like glucose-1-P and co-chromatographed with an authentic sample of the phosphoric ester). When the product under study was subjected to mild acid hydrolysis (0.1 N HCl - 100ºC for 10 min) or to alkaline phosphatase (Escherichia coli, Sigma) digestion, paper

TABLE I - Measurement of [^{14}C] Glucose Incorporation in Potato Starch Granules

Glucosyl donor	[^{14}C]Glucose incorporation into starch	
	Treatment of grain	
	None	8 M urea
	cpm	cpm
ADP-[^{14}C]glucose (812 pmol; 45,300 cpm)	1,690	8,616
UDP-[^{14}C]glucose (675 pmol; 176,000 cpm)	2,542	14,500

Reaction mixtures and assay were as described in Materials and Methods.

chromatography in solvent B showed in both cases two main radioactive peaks which were identified as glucose and maltose. Incubation of the unknown labeled material with glycogen and potato phosphorylase (16) was successful in incorporating part of its ^{14}C label into glycogen. This unexpected formation of [^{14}C]glucose-1-P, a conclusion we reach after discarding any contamination (see Materials and Methods), was attributed to the presence in starch grains of an enzymatic activity similar to that described by Randall and Tolbert (17). Incubation of ADP-[^{14}C]glucose with urea treated starch grains previously digested with α-amylase, resulted in the formation of [^{14}C]glucose-1-P and small amounts of labeled glucose; these findings suggest the occurrence in starch grains of an enzyme of the pyrophosphatase type, that seems to be liberated upon α-amylolysis of the grain, and that acts on linkages of the glucose-P-P-X or glucose-glucose-P-P-X types.

Mild Acid Hydrolysis

When labeled disrupted starch grains were hydrolyzed under very mild acid conditions (0.01 N HCl - 100ºC for 3 min) and precipitated with

methanol, radioactivity was almost completely released into solution. Analysis of the label by paper chromatography in solvent D showed the presence of labeled glucose and maltose. Sometimes, maltotriose and higher malto-oligosaccharides also appeared on the chromatogram in very small amounts. Treatment of radioactive intact starch grains under the same conditions did not liberate any detectable radioactivity into solution. Release of sugars under the above mentioned conditions is peculiar of the pyrophosphate linkage (18).

Treatment with Alcohol/NH_3

When labeled disrupted starch granules were submitted to the action of alcohol/NH_3 (80/20, v/v) at room temperature for 20 h, most of the radioactivity was released into solution. The solubilized product, electrophoresed in buffer A, appeared negatively charged. Paper chromatography in solvent A showed most of the label in a broad zone corresponding to that of glucose-1,2-cyclic phosphate (13). Mild acid treatment (0.1 N HCl - 100ºC for 10 min) of the latter, followed by alkaline phosphatase digestion liberated neutral ^{14}C labeled compounds, which were identified as glucose and maltose by paper chromatography in solvent D. Formation of cyclic diester derivatives upon alkaline hydrolysis is typical of pyrophosphate bonds attached to glucose or maltose (13).

Upon α-amylase digestion of the unattacked residue after the ammonia treatment, [^{14}C]glucose-1-P was obtained, indicating an incomplete action of NH_3 apparently due to the insolubility of starch grains in alcohol/NH_3.

TABLE II - Digestion of the Insoluble Labeled Material by Proteolytic Treatments

Treatment	Methanol insoluble	Methanol soluble
	cpm	cpm
-	10,700	75
Pronase	1,089	9,638
Subtilisin	543	9,938
Chaotropase	3,118	6,065

Treatment with Proteolytic Enzymes

Incubation of urea disrupted ^{14}C labeled starch granules with pronase (protease type V, Sigma), subtilisin (protease type VII, Sigma) or chaotropase (Calbiochem) released most of the radioactivity into solution (Table II). The solubilized material was shown by electrophoresis in buffer A to be negatively charged. The mild alkaline treatment (paper chromatography in solvent A) of the proteolytic digests led to the formation of cyclic phosphate derivatives (13), as judged by their mobilities in paper chromatography in solvent A. These products were resistant to alkaline phosphatase degradation, unless they were previously treated with 0.1 N HCl at 100ºC for 10 min, conditions that cause the opening of the cycle, most likely at position 1 of the sugar ring. The anionic properties of the resulting compounds were then lost by alkaline phosphatase degradation, which produced [^{14}C] glucose mainly, and also [^{14}C] maltose and higher labeled oligosaccharides in lower proportions.

Digestion of labeled intact grains with proteolytic enzymes did not release any detectable radioactivity into solution.

DISCUSSION

Incubation of disgregated potato starch grains in the presence of 8 M urea with ^{14}C labeled ADP-glucose or UDP-glucose caused an enhanced incorporation of [^{14}C] glucose into starch over that of untreated grains. This increase was not associated to an enlargement of the amylose or amylopectin glucosidic chains, as was observed for intact grains (15). [^{14}C] Glucose incorporation in disrupted starch grains brought about the formation of a compound in which labeled glucose or maltose units seemed to be linked to a protein(s) of the granule through a pyrophosphate linkage. Presumably, ADP-glucose or UDP-glucose donates a glucose-1-P group to an endogenous protein phosphate acceptor as shown by the formation, from labeled urea treated grains, of [^{14}C]glucose-1-P by a pyrophosphatase rendered active after α-amylolysis. The probable existence of a pyrophosphate bridge in the labeled compound formed was indicated by the ability to produce cyclic phosphate esters upon mild alkaline treatment and by the observed sensitivity to hydrolysis at pH 2.0. These results were in agreement with data obtained by Tomos and Northcote (19) in paramylon synthesis.

At present, it has not yet been established whether we were dealing with an enzyme activated upon disgregation of the grains or it was the well known starch synthetase which acts in a different way under conditions that caused the breakdown of the structure of the grain. The real meaning of the reaction found is presently unknown, although its close relation to starch biosynthesis appeared highly probable.

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