A PHOSPHORYLASE INVOLVED IN STARCH BIOSYNTHESIS

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SUMMARY: A novel phosphorylase, which has no requirement for primer addition, has been isolated from Solanum tuberosum. The properties of this enzyme were found to differ from those of the classical potato phosphorylase. The results are discussed in terms of the possible role of this enzyme in starch biosynthesis.

No suitable soluble primers have yet been demonstrated for amylopectin and amylose biosynthesis in plant cells. Illingworth, Brown and Cori (1), working with muscle phosphorylase , found that this enzyme could polymerize glucose-1-phosphate in the absence of any added primer, although at a very low rate. This "de novo" synthesis by an α-glucan phosphorylase was however questioned (2,3) and Nikuni et al (4) showed conclusively that potato phosphorylase could not catalyze polysaccharide formation in the absence of added primer, at least at a reasonable rate.

In a previous paper (5), we described the formation of a soluble amylopectin-like polysaccharide when slices of potato sprouts or tubers were incubated in the presence of 0.1 M glucose-1-P. We showed that this formation was neither due to "de novo" induction of the enzymes related to α-glucan formation nor to their activation. Several properties of the reaction (5) prompted us to suspect that this polysaccharide could be formed by a phosphorylase.

This communication describes the "de novo" formation of the amylopectin-like polysaccharide in a cell free system and provides evidence that this polysaccharide is formed by a novel phosphorylase, different from the classical enzymes described up to now, (4), (6), (7), (8). This new enzyme could be a glycoprotein with the glycosidic component acting as a primer.

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Materials and methods: Experiments were carried out with potato tubers, Kennebec variety. These were grated with a glass grater, and 0.1 ml of mercaptoethanol was added for each 25 ml of suspension. The suspension was filtered through cheese cloth and centrifuged for 10 minutes at 15,000 g at 0°-4°.The supernatant was dialyzed overnight against 4 l of 0.001 M citrate buffer (pH 6.2) and the dialyzed extract was centrifuged for 10 minutes at 15,000 g. The supernatant (crude extract) was precipitated with solid ammonium sulphate between 0-40% saturation (AS 40), the precipitate dissolved, and this fraction and the crude extract were dialyzed overnight as described and used as enzyme source. The AS40 fraction was less stable than the crude extract.

Enzyme assay: The reaction mixture contained, in a final volume of 40 µl, 1 µmole of citrate buffer (pH 6.2), 1 µmole of G-1-P, 10 µl of enzyme (0.1 mg of protein), and, when indicated, NaF (0.25 µmoles). The mixture was incubated for 45 min at 37°. The reaction was stopped either by addition of 50 µl of 0.2% I_2-2% KI (when polysaccharide formation was measured) or by dilution with water (when inorganic phosphate was estimated). The latter was determined by the method of Fiske and SubbaRow (9).

TABLE I

Change in phosphorylase activity with temperature and storage

The assay system was as described in Methods. The dialyzed "crude extact" was used as enzyme source. The polysaccharide formed in the absence of added primer, was measured with I_2-KI and the inorganic phosphate was measured as described. An OD of 0.1 units was equivalent to 0.085 µmoles of glucose.

Treatment	Polysaccharide formed		Phosphorylase activity µmoles of Pi formed	
	OD 560 mµ	µmoles of glucose	without added primer	with amylose*
-	0.52	0.44	0.45	0.45
Heated at 55° for 5 min	none	0	none	0.40
Kept one week at 4°C	0.43	0.37	0.38	0.40
Kept one week at 15°	0.08	.06	0.05	0.36

*-These values correspond to classical phosphorylase activity.

When classical phosphorylase activity was assayed, the same incubation mixture was used except for the addition of amylose (0.25 mg). The reaction was stopped by dilution and inorganic phosphate was determined. The presence of the branching enzyme (Q enzyme) was assayed on amylose by the method of Krisman (10).

Results: The results showed (Table I) that the extract had a phosphorylase activity which disappeared at 55°, in contrast to the classical phosphorylase activity. It is interesting to point out that one of the methods used to purify the classical phosphorylase (4) was heating for 10 min at 55°C. There was also a very good correspondence (Fig 1) between

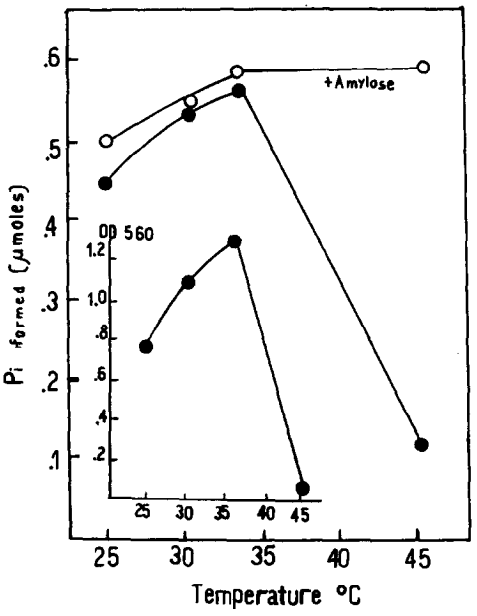

Fig. 1
Effect of temperature on enzyme activity.
Incubation mixtures were as described in Methods, except for the incubation temperatures, which were the indicated.

polysaccharide formation and inorganic phosphate appearance, in the absence of primer. When electrophoresis on acrylamide gel was performed on this preparation (Fig 2), five bands of phosphorylase activity were detected when glycogen was included, according to Stegermann's method (12). This is in agreement with the data obtained by Aimi and Murakami (13). However, when the addition of primer was omitted, only one band of activity remained. When the enzyme lost almost 85% of its activity on storage (Table I), the electrophoretical analysis (Fig 2a) indicated

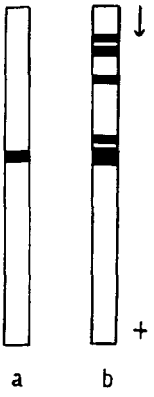

Fig. 2
Analytical acrylamide gel electrophoresis.
Performed according to Davis and Ornstein (11). The gel was cut into halves; one half was stained with Amido Black and the other was incubated with citrate-buffer and G-1-P for 13 hours. a) Enzyme (crude extract) without primer inclusion. b) Enzyme (crude extract) with glycogen (0.1%) included during the run.

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only scant formation of polysaccharide. These data lend support the idea that the primer is bound to the protein. The product formed was a branched polysaccharide, identical to the intracellular α-glucan already described by us (5).

We tried to determine if this "de novo" process proceeded by a mechanism similar to that described for glycogen in Aerobacter aerogenes (14) or by the classical phosphorylase-branching enzyme mechanism. To elucidate this, we looked for a number of substances which could affect the branching activity without affecting polysaccharide formation. We found that ATP and $MgCl_2$, at certain concentrations, inhibited the activity of the Q-enzyme but not the amylose forming capacity of the phosphorylase. Hence we assayed the effect of both substances on our enzymatic system (Table II). The results show that when branching activity was supressed, amylose was formed. Also, when the system was free of branching enzyme, (AS 40 Supernatant), amylose was the only product of the reaction. This supports the view that our polysaccharide is formed by the combined action of phosphorylase and Q-enzyme.

The activity of the new phosphorylase (as measured by polysaccharide formation) was not affected by the addition of inorganic phosphate even at a concentration twice that of glucose-1-phosphate.

Discussion Although the enzymes involved in amylose and amylopectin biosynthesis are known (15), (16), it is unlikely that they are the only ones responsible for "de novo" starch biosynthesis, since they require a

TABLE II

Efect of inhibitors on branching of the polysaccharide
The incubation mixture was free of any added primer. The assay conditions were as described.

Enzyme	Addition	Polysaccharide formed		Q-Enzyme
		Amylose Type	Amylopectin Type	
		OD 650	OD 560	ΔOD 650
AS40*				
	None	-	0.53	0.28
AS40	ATP (0.25 umole)	0.60	-	0.10
AS40	$MgCl_2$ (1 umole)	0.61	-	0.0
AS40 Supernatant	None	0.30	-	0.0

*-Similar results were obtained when "Crude extract" was used as enzyme source.

preformed -glucan or oligosaccharide for their chain elongating activity. Several workers (17) (18) discussed the possibility that a phosphorylase could be involved in the process. This paper demonstrates that amylose and amylopectin could be formed by a soluble phosphorylase-Q enzyme system present in potato tubers whithout any primer addition. This "de novo" formed polysaccharide is a very good primer for starch synthetase (5).

The described system behaved like a phosphorylase, forming amylose at a very good rate from glucose-1-phosphate, and amylopectin when combined with branching enzyme. The whole system (phosphorylase-Q enzyme) was sensitive to ATP and $MgCl_2$, which inhibited the Q-enzyme. Since ATP and Mg^{++} are constituents of the cells this may be of physiological importance.

Phosphorylase from plant sources have been studied extensively (4), (8). None of them was found to act at a reasonable rate without primer addition. This may be due to the lability of the non primer requiring phosphorylase, which becomes inactive during the purification processes. A possibility which cannot yet be discarded is that the loss of a primer fraction transforms our phosphorylase into the primer requiring one.

The detailed properties of the new system will be published elsewhere.

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