A new transglucosidase found in potatoes
by Clarence A Ryan

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Montana State University
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Abstract:
A new transglucosidase, found in potatoes, is reported, The enzyme transfers the non-reducing glucose from isomaltose to acceptor molecules of maltose, maltotriose and1 higher molecular weight maltodextrins forming linear primers for the starch phosphorylase reaction, The enzyme was identified by activation of starch phosphorylase by forming primers for the latter enzyme. This activation was noted by both an increased formation of the amylose-I2 blue complex and increased release of inorganic phosphate when isomaltose was added to the partially purified enzyme system containing starch phosphorylase. The enzyme preparation also contained chromatographically detectable impurities of glucose, maltose and maltotriose.

The transglucosidase mechanism was identified by (1) it's role in furnishing substrates for the starch phosphorylase reaction, (2) incorporation of C14 from glucose-U-C14 into the isomaltose moiety after equilibration with the enzyme and isomaltose for 48 hours, and, (3) by the incorporation of Cl4 from isomaltose-U-Cl4 into maltodextrins with the subsequent release of glucose-Cl4 into the incubation media when the enzyme was incubated with isomaltose-U-C14, maltose and glucose-1-phosphate .

The proposed mechanism is as follows: Isomaltose + Enzy--------- Enzyme-Glucose + Glucose
Enzyme-Glucose + Maltose --------- Maltotriose + Enzyme / starch Maltotriose + Glucose-1-phosphate
phosphorylase amylose+ Pi

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ABSTRACT

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The enzyme was identified by activation of starch phosphorylase by forming primers for the latter enzyme. This activation was noted by both an increased formation of the amylose-I_2 blue complex and increased release of inorganic phosphate when isomaltose was added to the partially purified enzyme system containing starch phosphorylase. The enzyme preparation also contained chromatographically detectable impurities of glucose, maltose and maltotriose.

The transglucosidase mechanism was identified by (1) it's role in furnishing substrates for the starch phosphorylase reaction, (2) incorporation of C^{14} from glucose-U-C^{14} into the isomaltose moiety after equilibration with the enzyme and isomaltose for 48 hours, and, (3) by the incorporation of C^{14} from isomaltose-U-C^{14} into maltodextrins with the subsequent release of glucose-C^{14} into the incubation media when the enzyme was incubated with isomaltose-U-C^{14}, maltose and glucose-1-phosphate.

The proposed mechanism is as follows:

$$\text{Isomaltose} + \text{Enzyme} \longleftrightarrow \text{Enzyme-Glucose} + \text{Glucose}$$

$$\text{Enzyme-Glucose} + \text{Maltose} \longleftrightarrow \text{Maltotriose} + \text{Enzyme}$$

$$\text{Maltotriose} + \text{Glucose-1-phosphate} \xleftrightarrow[\text{phosphorylase}]{\text{starch}} \text{Amylose} + P_i$$

I. INTRODUCTION

The normal metabolism of a large number of plants involves the storage of carbohydrate material as a readily available energy source. Many of these plants store this carbohydrate material in the form of starch, a large polymer of alpha-D-glucose, which can be fractionated into two molecular types called amylose and amylopectin. The amylose fraction is linear and only alpha 1-4 bonds between the glucose units have been identified in the molecule. There is however an anomalous bond present which can be detected by Z-enzyme (1). The nature of this bond is unknown due to the inactivity of the enzyme on synthetic substrates (2). The molecular size of amylose may vary somewhat from an average of about 300 glucose units (3,4) although no length limiting factor has yet been discovered. The lower limit of the molecular size is difficult to define. Amylose and amylopectin both form colored complexes with iodine due to the quantitative binding of I_2 inside of the helix of the starch molecule (5). One full turn of the helix involves about six glucose units and binds approximately 0.21 g. of iodine per complete turn. The color of this complex is brown for amylopectin and blue for amylose. The blue complex of amylose persists down to about a 16 glucose unit chain called a maltodextrin. As the chain length decreases the color changes, becoming light red at approximately 8 glucose units (6). It would seem logical to consider this 8 unit chain the lower limit of the size of the amylose molecule although lower molecular weight straight chain oligosaccharides are usually found associated with this fraction.

Amylopectin possesses a branched, treelike structure. The straight chained portions of the molecule between branches are bonded alpha 1-4 and the branching points involve an alpha 1-6 bond. The average distance between branching points is about 10-12 glucose units (7). The average amylopectin molecule is much larger than the average molecule of amylose, having a D.P.[1] of about 1300 glucose units (4,7,8).

The percentages of the two fractions, amylose and amylopectin, vary with the source of the starch. Starches generally contain a larger percentage of amylopectin than amylose, usually from 70% to 90% of the total starch.

The synthesis of starch in the plant is not well understood. In 1940, Hayne discovered an enzyme in the potato (9) that required alpha-D-glucose-1-phosphate and suitable maltodextrins as substrates for the synthesis of amylose. It utilizes the energy present in the hemiacetal phosphate bond of glucose-1-phosphate to synthesize an alpha 1-4 bond. This enzyme catalyzes the transfer of the glucose of glucose-1-phosphate to suitable acceptor molecules, the maltodextrins, to form longer linear amylose type molecules with the simultaneous release of inorganic phosphate into the medium. The enzyme, starch phosphorylase, requires maltotriose or a larger maltodextrin as the acceptor molecule for the glucose moiety. Neither maltose nor free glucose will act as a substrate for this enzyme (10). The equilibrium of the starch phosphorylase reaction is 75.6 to 24.4 in favor

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1. D. P. - Degree of polymerization

of synthesis of polysaccharide at a pH of 6.96 (11).

The phosphorylase reaction is, in fact, a transglucosidase reaction, that is, a reaction that involves the transfer of glucose from one moiety to another, conserving the energy in the original bond. The energy of the bond in the original substrate, e.g. glucose-1-phosphate, or between two sugar moieties, is conserved in an enzyme-substrate compound and this energy again conserved in an acceptor-glucose bond. This is a double displacement reaction and the configuration in the glucose-acceptor molecule is the same as in the original compound.

An enzyme from the potato has been reported that transfers two or more units from maltodextrins to suitable acceptors to give straight chained dextrins (12). The substrate appears to be maltotriose or larger maltodextrins and only alpha 1-4 bonds are synthesized. The enzyme has been called the disproportionating or D enzyme. Since glucose and maltose will act as acceptors, degradation of starch proceeds when these acceptors are present in appreciable amounts. It is probable that this enzyme synthesizes amylose only in the young plant when large amounts of small maltodextrins are present or when hexokinase reduces the glucose concentration to a minimum (13).

A third starch synthesizing enzyme, Q enzyme, is also a transglucosidase (14). It transfers straight chained amylose fragments to the 6 position of acceptor maltodextrins forming branched components or amylopectin like molecules. This enzyme requires a maltodextrin of at least 42 glucose units in length as the substrate (15,16).

A recent paper has suggested that the formation of starch involves glycogen as a precursor to both amylose and amylopectin but no experimental evidence of the enzyme systems has been offered (17).

To the present time no evidence regarding the origin of the primers for phosphorylase or D enzyme has been found. It has been suggested that transglucosidases may be involved. Enzymes of this type have been found in bacteria. An enzyme found in Pseudomonas saccharophila (18) catalyzes the formation of an amylose-like molecule from sucrose. The reaction is as follows:

$$n \text{ Sucrose} \longleftrightarrow (\text{Glucose})_n + n \text{ Fructose}$$

A similar enzyme from Escherichia coli (19) catalyzes the formation of primers from maltose.

$$n \text{ Maltose} \longleftrightarrow (\text{Glucose})_n + n \text{ Glucose}$$

Sucrose and maltose are both known constituents of plants. The synthesis of sucrose is known to occur in wheat germ by three pathways(20).

(1) UDPG[2] + Fructose $\longleftrightarrow$ Sucrose-phosphate + UMP[3]

(2) UDPG + Fructose-6-phosphate $\longleftrightarrow$ Sucrose-phosphate + UDP

(3) UDPG + Fructose $\longleftrightarrow$ Sucrose + UDP

Maltose is a common constituent of plants and is generally believed to be a result of alpha and beta amylase action on starch. No enzymes have been reported which synthesize maltose in the plant. However, it has been

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2. UDPG -- Uridine Diphosphoglucose
3. UMP -- Uridine Monophosphate

reported that maltose-U-C^{14} is utilized in the tobacco leaf for starch synthesis (21). The hypothesis suggested a conversion to sucrose, and then to starch.

Maltose is known to be synthesized by a maltose phosphorylase found in Neisseria meningitidis (22). The enzyme utilizes beta-D-glucose-1-phosphate and glucose as substrates.

$$\text{Beta-D-Glucose-1-phosphate} + \text{Glucose} \rightleftharpoons \text{Maltose} + P_i$$

Both sucrose and maltose require an activated intermediate for their synthesis. This leaves the investigator many paths to investigate. The problems in elucidation of the origin of starch primers can be seen to be very complex.

In the present paper the presence of a transglucosidase in the potato is reported which catalyzes the transfer of the non-reducing terminal glucose of isomaltose to maltose and higher maltodextrins, thus building linear type molecules which can assume the role of primer for starch phosphorylase. A mechanism for this reaction has been proposed from the experimental evidence.

A synthesis of isomaltose from monosaccharides has not been reported at the present time; however, the action of the enzyme described in this paper would suggest the presence of an isomaltose synthesizing enzyme in the potato.

Isomaltose has not been previously considered as a course of the primers for starch phosphorylase. The only reports of isomaltose in plant metabolism has been from the breakdown of starch and this was in very low

yield i.e. 1.0 to 2.0%. The presence of sucrose in most young plants led workers to believe that this was probably the source of amylose via a transglucosidation reaction. This is still speculation and may occur in some plants. The absence of isomaltose does not rule the disaccharide out of a possible role in 'primer' synthesis since we know in a "steady state" a build up of an intermediate may not occur. Nevertheless isomaltose has been overlooked as an intermediate. This paper would suggest that a detailed investigation of 'primers', especially in the young plant, is in order.

II. EXPERIMENTAL

1. Preliminary Observations.

During an investigation of starch phosphorylase in a centrifuged, 60 hour dialyzed fraction of potato juice, it was found that the addition of C.P. D-glucose to a mixture of alpha-D-glucose-1-phosphate and an aliquot of the juice increased the rate of formation of the blue amylose-I_2 complex normally formed by the starch phosphorylase reaction (Fig. 1).

The enzyme was prepared by first thinly slicing peeled Russet potatoes into a sodium dithionite solution (7 g./1), and soaking them for 10 minutes at room temperature. The slices were pulped in a Waring blendor and the juice from the pulp was expressed through six thicknesses of cheese cloth into a cooled beaker. The juice was centrifuged in a refrigerated centrifuge at 5° C. at 10,000 x g. and the residue discarded. This juice was dialyzed against cold running tap water for 60 hours. This treatment destroyed much of the starch phosphorylase and precipitated large amounts of protein material. The dialyzed fraction was centrifuged at 10,000 x g. The supernatant was cooled and used as the crude enzyme preparation.

The enzyme assay contained the following reagents: 0.5 ml. of the enzyme preparation; 0.5 ml. of 0.25 M. citrate buffer pH 6.0; 10 μM. of glucose-1-phosphate; and 5 μM. of glucose. A similar assay was made without the addition of glucose. Final volumes were made to 2.5 ml. with distilled water. A 1.0 ml. aliquot was taken from the mixture after a 15 minute interval and added to 2.0 ml. of I_2 in KI solution (23). The I_2 - KI solution was made by diluting 1.0 ml. of a 0.5 M. KI, 0.05M. KCl solution containing

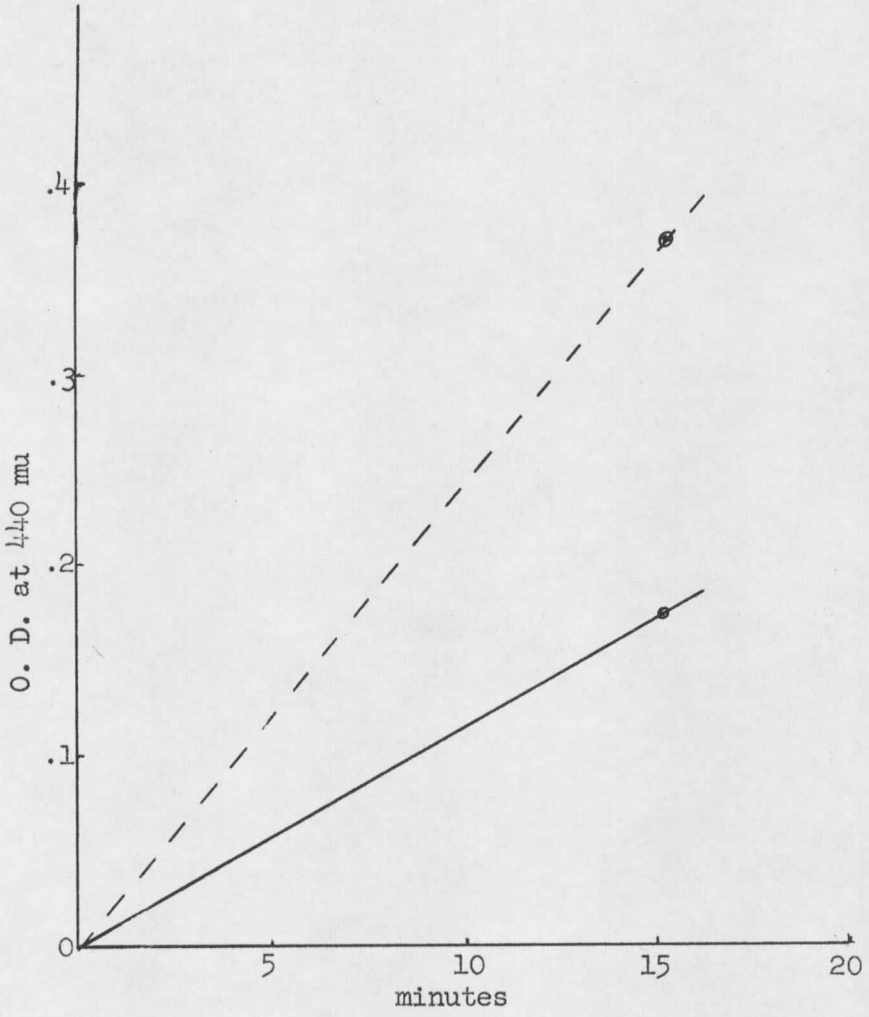

Figure 1. The effect of the addition of glucose on the formation of the blue I_2-amylose complex formed by the starch phosphorylase reaction. The enzyme preparation also contained primer molecules for the reaction as impurities.

Legend: - - - - - 5 μM. of glucose added to assay.
_____ no glucose added to assay.

2.00 g. of I_2 per liter to 50 ml. with water. The color, which is stable for 10 minutes, was recorded immediately at 440 mu on a Beckman Model B spectrophotometer. These results were reproducible. This assay is but a qualitative measurement of a rate of polysaccharide formation and tells little about the concentration. This is because one long amylose molecule will complex more I_2 than will two shorter molecules containing the same total number of glucose units. The only conclusion that could be drawn from the observed increased in the rate of formation of the blue I_2 complex was that more primer molecules must have been present in the assay mixture due to the addition of glucose. This suggested the incorporation of glucose into polymers. No known enzyme systems are capable of catalyzing this reaction under these conditions.

The glucose, when chromatographed on paper, did not reveal the presence of primers of starch phosphorylase as impurities. A typical chromatogram was made by spotting 100 µg. of glucose one inch from one end of a strip of Whatman #1 filter paper, 1 1/2 inches wide by 12 inches long. After air drying the spot, the chromatogram was suspended inside a bell jar with the end containing the spot immersed to a depth of about 10 mm. into a n-butanol, pyridine, water mixture (6:4:3 v/v). This chromatogram was dried and re-ascended to get better separation of the sugars. The chromatogram, after drying, was sprayed with a glucose-1-phosphate-starch phosphorylase spray reagent (10) and incubated in a moist atmosphere for fifteen minutes. The chromatogram was then sprayed with the I_2 in KI, KCl solution that was used in the amylose-I_2 color assay mentioned above. Any primer molecules

present as impurities would have resulted in the formation of blue spots due to the action of the starch phosphorylase which forms amylose type molecules from the primers. Maltotriose is the smallest primer that can be utilized by starch phosphorylase for synthesis of the larger molecules (10). This method will detect microgram quantities of primers.

The phosphorylase was prepared from crude potato juice by a modification of the method of Baum and Gilbert (24). The crude juice was prepared in a manner similar to that of the new enzyme preparation except that the juice was not dialyzed. Instead, after centrifuging the cooled, crude juice at 10,000 x g, 0.28 ml. of 50% ethanol (w/v) in 0.01 M. sodium citrate buffer, pH 6.0, was added per ml. of the supernatant. This mixture was centrifuged at 10,000 x g and the precipitate discarded. The supernatant liquid contained active starch phosphorylase and was used in spraying the chromatograms. This preparation located primer impurities in C. P. maltose when used under the conditions of the previous experiment.

2. The effect of the citrate ion in phosphate analysis using a molybdate reagent.

The increase in the rate of formation of the blue I_2 complex due to glucose was not consistently accompanied by release of inorganic phosphate as measured by the method of Sumner (25). Any theory explaining the observed glucose stimulation of starch phosphorylase action requires the release of inorganic phosphate, (P_i), since polysaccharide formation could not be detected without the addition of glucose-1-phosphate to the incubation mixture.

The inconsistency of the P_i release was thought to be caused by the

reagents. However, when standard phosphate solutions were analyzed in the presence of increasing concentrations of the 0.25 M. citrate buffer, pH 6.0, which was used for the enzyme assays, it was found that the citrate ion quantitatively bound the molybdate ion in solution independently of the phosphate present (Fig. 2). It was shown that the addition of 0.3 ml. of 0.25 M citrate buffer to a phosphate assay barely inhibited the formation of the blue phosphomolybdate color. During the original enzyme assays, 0.5 ml. aliquots, when taken from the enzyme assay mixture, were equal to 0.125 ml. of the buffer (Fig. 2). This explained the wide variations in the phosphate assays.

To correct for this phenomenon the percent of ammonium molybdate was raised from 2.5% to 7.5%. A series of known phosphate solutions were assayed with increasing aliquots of the citrate buffer to find if the increased percent of ammonium molybdate would correct the situation (Fig. 3). No effect due to the buffer was observed when as much as 0.5 ml. of the buffer was added. This was four times the amount generally used in the enzyme assays for P_i.

The 7.5% ammonium molybdate solutions gave reproducible P_i analyses during the remainder of the laboratory work.

3. The enzyme preparations.

The early evidence of the new enzyme in potato juice had shown the enzyme to be stable toward prolonged dialysis. This treatment precipitated large volumes of protein material during the first 12 to 18 hours. It was decided to fractionate the 18 hour dialysate with ammonium sulfate in order