Biosynthesis of Starch in Spinach Chloroplasts*

Hara Prasad Ghosh and Jack Preiss

ABSTRACT: A soluble-enzyme system transferring glucose from adenosine 5′-diphosphate glucose (ADP-glucose) to a primer to form α-1,4-glucosyl linkages has been partially purified from spinach chloroplast. Of a number of sugar nucleotides tested only ADP-glucose and deoxy-ADP-glucose could serve as the glucosyl donor. The acceptor could be an α-1-4-glucan or oligosaccharide of the maltodextrin series. The enzyme has a *p*H optimum of 8.5 and is inhibited by *p*-mercuribenzoate. Glycolytic intermediates had no stimulatory effect on the transglucosylase. The primer requirements of this transferase are compared to the primer requirements of other plant ADP-glucose–α-1,4-glucan transferases.

The biosynthesis of starch was first shown by Leloir *et al.* (1961) to take place by the transfer of the glucosyl moiety of UDP-glucose[1] to an acceptor. Subsequently, Recondo and Leloir (1961) reported that ADP-glucose was a better glucosyl donor than UDP-glucose. It also was found that in ripening rice grains the transfer of glucose to the starch primer occurred more rapidly from ADP-glucose than UDP-glucose (Murata *et al.*, 1964; Akazawa *et al.*, 1964). In the leaves of higher plants starch is known to be synthesized and accumulated in the chloroplast (Badenhuizen, 1963). Murata and Akazawa (1964) have recently shown that in leaves starch is formed from ADP-glucose only. In all of these cases the ADP-glucose–starch transglucosylase activity was associated with starch granules and therefore particulate. Frydman and Cardini (1964a) obtained a soluble preparation from sweet corn which transferred glucose from ADP-glucose in phytoglycogen, a soluble polysaccharide related to amylopectin. They have also recently (Frydman and Cardini, 1964b) shown that an enzyme present in the 18,000 × *g* supernatant fluid prepared from extracts of tobacco leaves or from potato tubers transferred glucose from ADP-glucose to an α-1-4-glucan primer. In a preliminary communication (Ghosh and Preiss, 1965) the presence of a soluble ADP-glucose–starch transglucosylase in spinach chloroplast had been reported. The present communication reports in detail the partial purification and properties of the ADP-glucose–α-1,4 glucan-α-4-glucosyl transferase in spinach chloroplasts.

Recent preliminary notes by Doi *et al.* (1964) and Murata *et al.* (1965) also have shown the presence of a soluble ADP-glucose–α-1-4-glucan transglucosylase in spinach chloroplasts and in glutinous rice grain extracts, respectively.

Experimental Procedure and Results

Substrates. ADP-[^{14}C]glucose and deoxy-ADP-[^{14}C]glucose were prepared from ATP and deoxy-ATP plus [^{14}C]glucose-1-P and *Arthrobacter* ADP-glucose pyrophosphorylase (Shen and Preiss, 1965b). CDP-[^{14}C]glucose was prepared from CTP, [^{14}C]glucose-1-P, and the *Arthrobacter* ADP-glucose pyrophosphorylase (which can synthesize CDP-glucose in low yields). UDP-[^{14}C]glucose and TDP-[^{14}C]glucose were prepared from their respective triphosphate, [^{14}C]glucose-1-P, and ammonium sulfate fractions of the protamine supernatant fluid of the *Arthrobacter* extract (Shen and Preiss, 1965b). The radioactive sugar nucleotides were isolated by column chromatography and desalted by adsorbtion onto Norit A and elution therefrom (Shen and Preiss, 1965b; Roseman *et al.*, 1961). Nonradioactive sugar nucleotides were synthesized chemically by a modification (Kochetkov *et al.*, 1962) of the procedure of Roseman *et al.* (1961). The amylose used was a soluble corn amylose preparation prepared by the butanol fractionation procedure of Schoch (1957). Amylopectin was obtained from Calbiochem (Los Angeles, Calif.). Rabbit liver glycogen was obtained from Mann Research Laboratories, Inc. (New York). *Escherichia coli* glycogen was a gift from Dr. I. H. Segel, University of California, Davis. Starch granules prepared according to the method of Leloir *et al.* (1961) were obtained from Dr. M. Mazelis and Mr. M. Tishel, University of California, Davis. [^{14}C]Glucose-1-P and GDP-[^{14}C]glucose was obtained from International Chemical and Nuclear Corp. (City of Industry, Calif.). Laminaribiose was obtained from Dr. D. Feingold, University of Pittsburgh, Pittsburgh, Pa. and sophorose was obtained from Dr. G. Ashwell of the National Institute of Health, Bethesda, Md. All other nonradioactive compounds were obtained from commercial sources.

* From the Department of Biochemistry and Biophysics, University of California, Davis. *Received February 23, 1965.* This work was supported by a grant (AI-05520) from the U.S. Public Health Service.

[1] Abbreviations used in this work: UDP, ADP, CDP, TDP, GDP, IDP, the 5′-diphosphates of uridine, adenosine, cytidine, deoxyribosylthymine, guanosine, and inosine, respectively; AMP, adenosine 5′-phosphate; ATP, CTP, the 5′-triphosphates of adenosine and cytidine, respectively; GSH, glutathione (reduced).

The following solvent systems were used in descending paper chromatography on Whatman No. 1 filter paper: solvent A, ethyl acetate–pyridine–water (3.7:1.0:1.15); solvent B, 1-butanol-pyridine-water (6:4:3); solvent C, 1-propanol–ethyl acetate–water (7:1:2). Paper electrophoresis was done in 0.05 M sodium tetraborate buffer, *p*H 9.4, in a GME electrophorator. Reducing sugars were detected on paper by silver nitrate technique (Anet and Reynolds, 1954). Protein concentration was measured by the method of Lowry *et al.* (1951).

Assay of Transglucosylase. ASSAY A. The reaction mixture, which contained 30 mμmoles of ADP-[^{14}C]-glucose (7.5×10^5 cpm/μmole), 10 μmoles of glycine-NaOH buffer, *p*H 8.5, 5 μmoles of KCl, 2 μmoles of GSH, 1 μmole of EDTA, 250 μg of soluble corn amylose, and the purified enzyme in a total volume of 0.2 ml, was incubated at 37° for 15 minutes. The reaction was stopped by the addition of 2 ml of 75% methanol containing 1% KCl. The mixture was then centrifuged and the amylose precipitate was washed twice with 2 ml of the methanol-KCl solution. The amylose was then dissolved in 1 ml of water and a 0.5-ml portion was counted with 10 ml of Bray's solution (Bray, 1960) in a liquid-scintillation counter. Controls containing no enzyme usually registered 10–25 cpm over background.

ASSAY B. The incorporation of glucose was also followed by the formation of ADP or deoxy-ADP. The reaction mixture, which contained 50 μmoles of glycine-NaOH buffer, *p*H 8.5, 25 μmoles of KCl, 10 μmoles of GSH, 5 μmoles of EDTA, 1.25 mg soluble corn amylose, and 0.15 μmole deoxy-ADP-glucose or ADP-glucose and the purified enzyme in a total volume of 1 ml, was incubated at 37° for 15 minutes. The reaction was terminated by heating in a boiling-water bath for 30 seconds. Portions of the reaction mixture were assayed for nucleoside diphosphate (Kornberg and Pricer, 1950).

Preparation of the Chloroplast and 105,000 × g Supernatant Fluid. Fresh spinach was obtained from the local supermarkets. Washed, deveined leaves (750 g) were homogenized in the cold with 1000 ml of a solution of 0.5 M sucrose containing 0.1 M phosphate buffer, *p*H 7.4, 0.01 M EDTA, and 0.005 M GSH in a Waring Blendor for 30 seconds. The nuclear debris and unbroken cells were removed by centrifuging at 300 × *g* for 1 minute and the chloroplasts were then isolated by centrifuging at 1000 × *g* for 10 minutes (Whatley and Arnon, 1963). The chloroplasts were washed with a solution of 0.5 M sucrose containing 0.01 M phosphate, *p*H 7.4, 0.002 M EDTA, and 0.005 M GSH. The washed[2] chloroplasts were suspended in 80 ml of 0.1 M Tris-succinate buffer, *p*H 7.0, containing 0.005 M GSH and 0.002 M EDTA, and disrupted in a French press at 20,000 psi. The broken chloroplasts were centrifuged at 105,000 × *g* for 90 minutes and the clear supernatant fraction was used as the enzyme source.

Purification Procedure. The 105,000 × *g* supernatant fluid was made to 40% saturation of ammonium sulfate by adding solid ammonium sulfate. After 15 minutes, the resulting suspension was centrifuged for 10 minutes at 30,000 × *g*. The precipitate was dissolved in a buffer containing 0.10 M Tris-succinate, *p*H 7.0, 0.005 M GSH, and 0.002 M EDTA, and dialyzed overnight against 1 liter of 0.02 M Tris-succinate, *p*H 7.0, 0.005 M GSH, and 0.002 M EDTA. To 12 ml of the dialyzed 40% ammonium sulfate fraction was added 6 ml of a calcium phosphate gel suspension (containing 22.5 mg of dry solid/ml). After 10 minutes the mixture was centrifuged at low speed. About 60–70% of the transglucosylase was adsorbed on the calcium phosphate gel. The transglucosylase activity was eluted from the gel twice with 6 ml of 0.05 M phosphate buffer, *p*H 7.5, containing 0.005 M GSH. Usually most of the enzymic activity came in the first eluate. The combined eluate was then made to 40% saturation of ammonium sulfate by adding solid ammonium sulfate. After 15 minutes the resulting suspension was centrifuged and the precipitate was dissolved in a buffer containing 0.1 M Tris-succinate, *p*H 7.0, 0.005 M GSH, and 0.002 M EDTA. It was then dialyzed overnight against 1 liter of 0.02 M Tris-succinate, *p*H 7.0, containing 0.005 M GSH and 0.002 M EDTA.

This procedure yielded a transglucosylase preparation purified about 9- to 12-fold over the 105,000 × *g* supernatant fraction (Table I). The enzyme contained no starch phosphorylase or ADP-glucose pyrophosphorylase activity. However a low level of amylase activity was detected. About 25% of the amylose was degraded by 0.26 mg of the gel fraction in 2 hours (measured by decrease in anthrone (Ashwell, 1957) reactive material that is precipitated by 75% methanol). Figure 1 shows the linearity of transglucosylase action with time and protein concentration.

Requirements for Transglucosylase Activity. Table II shows that glucose transfer is dependent on the presence of a primer and an enzyme. GSH, KCl, and EDTA consistently stimulated the enzymic activity 10–20%. If all three are omitted simultaneously, the activity is reduced to about 40%. The addition of α-amylase (2 μg) to the reaction mixture completely inhibited the incorporation of glucose from either deoxy-ADP-glucose or ADP-glucose.

Specificity of the Sugar Nucleotide Donor. The transglucosylase in both the 105,000 × *g* supernatant fraction and the purified enzyme was specific for ADP-glucose and deoxy-ADP-glucose. In assay A (Table III), neither UDP-[^{14}C]glucose, TDP-[^{14}C]glucose, GDP-[^{14}C]glucose, nor CDP-[^{14}C]glucose was able to substitute for ADP-[^{14}C]glucose. [^{14}C]Glucose-1-P alone or in the presence of 5′-AMP was also inactive. In assay B IDP-glucose, ADP-mannose, and ADP-galactose were inactive. Deoxy-ADP-glucose was about 50% as effective as ADP-glucose (Figure 2). Nucleoside diphosphate formation from either ADP-glucose or deoxy-ADP-glucose was absolutely de-

[2] Further washing of the chloroplast fraction did not affect the yield of transglucosylase activity obtained from the chloroplast extract.

TABLE I: Purification of the ADP-glucose–Starch Transglucosylase.

Fraction	Volume (ml)	Protein (mg/ml)	Total Units[a]	Specific Activity (units/mg)
105,000 × *g* supernatant fluid	80	7.5	54	0.09
Ammonium sulfate	12.6	5.8	51	0.70
Calcium phosphate gel + ammonium sulfate	16	1.1	21	1.2

[a] One unit = 1 μmole of [^{14}C]glucose transferred to amylose in 15 minutes under the conditions of assay A.

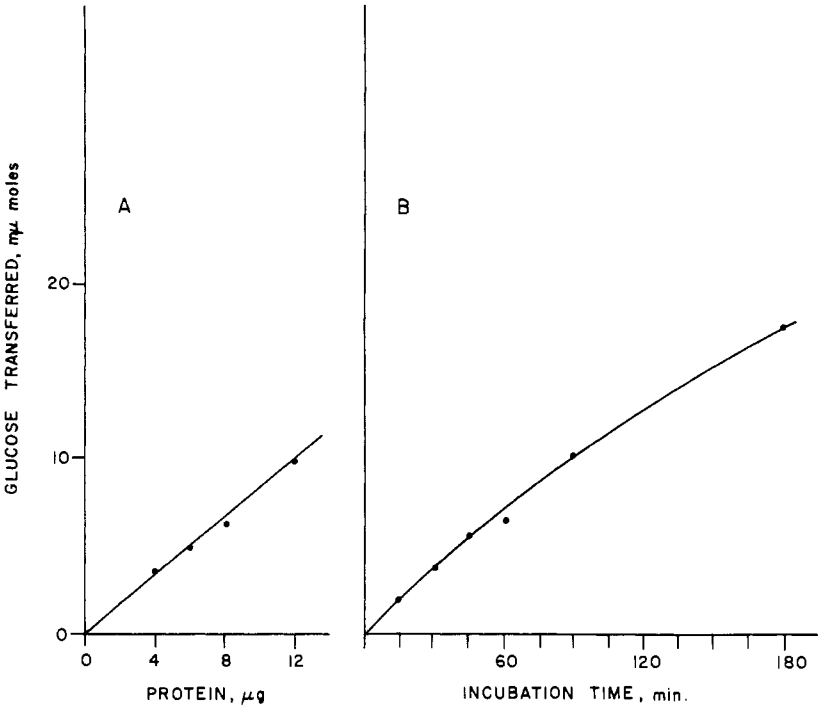

FIGURE 1: Linearity of transglucosylase activity with protein concentration and time. The conditions of the experiment were the same as in assay A.

pendent on the presence of an α-1→4-glucan primer and transglucosylase.

Specificity of Primer. Figure 3 shows that a number of α-1→4-polyglucans can serve as primer. The rate of transfer of glucose from ADP-glucose to soluble amylose or amylopectin was, however, faster than the rate of transfer to rabbit liver or *E. coli* glycogen. The comparison of the primers was made on a weight basis. Whole starch granules were about 50% as active as a primer, compared to soluble amylose. In order for starch granules to be active as a primer they had to be heated at 100° for 5 minutes. An aqueous suspension of the starch granules which were not heated had no priming ability. Different oligosaccharides were also tested for their priming activity. The reaction mixture as described in assay A was used except that various oligosaccharides were substituted for amylose as primer. The reaction mixture was incubated for 30 minutes at 37° and the reaction was stopped by heating in a boiling-water bath for 30 seconds. The mixture was diluted by adding 1.8 ml water, and 200 mg of solid Dowex 1-X8 (Cl) was added to adsorb the unreacted ADP-[^{14}C]glucose. The mixture was filtered through a Millipore filter and radioactivity of 0.5 ml of the filtrate, which contained the neutral primer together with the labeled product, was determined in 10 ml of Bray's solution (Bray, 1960) in a liquid-scintillation spectrometer. This assay will be referred to as assay C. The omission of enzyme or oligosaccharide acceptor resulted in the absence of radioactivity in the filtrate.

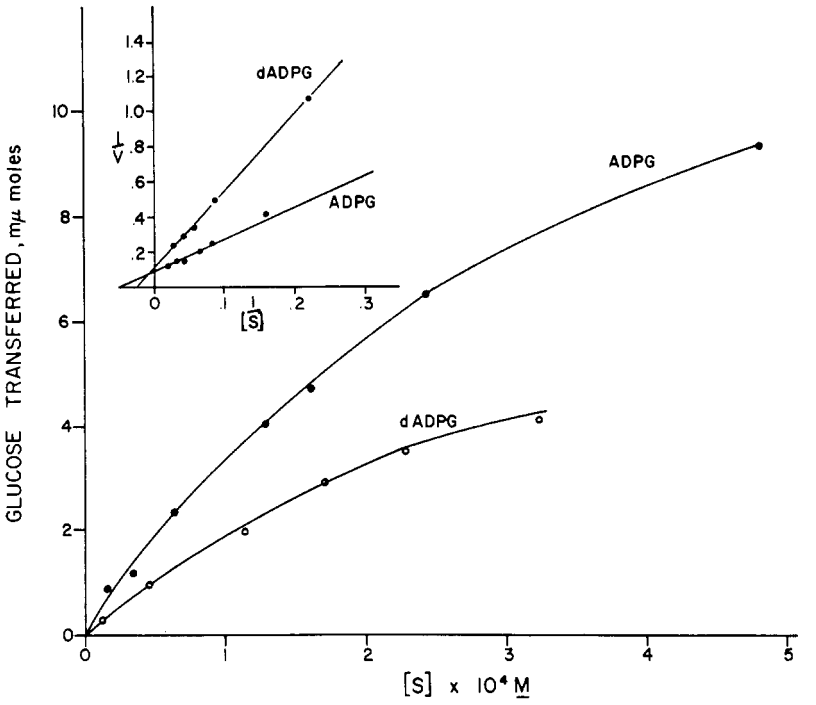

FIGURE 2: Dependence of reaction rate on substrate concentration. The conditions of the assay were the same as in assay A, with the indicated substrates at various concentration.

TABLE II: Requirements for Transglucosylase Activity.[a]

Omissions	[^{14}C]Glucose Incorporated (mμmoles)
None	12.3
-GSH	11.6
-KCl	11.0
-EDTA	10.3
-GSH, KCl, and EDTA	5.1
-Enzyme	<0.05
-Soluble corn amylose	<0.05
-Enzyme + denatured enzyme	<0.05

[a] The conditions of the experiment were those in assay A. The denatured enzyme was prepared by heating in a boiling-water bath for 30 seconds.

TABLE III: Specificity of the Glucosyl Donor.[a]

Glucosyl Donor	Quantity Used in Reaction Mixture (mμmoles)	Glucose Incorporated (mμmoles)
ADP-[^{14}C]glucose	38	28.2
Deoxy-ADP-[^{14}C]glucose	34	14.6
UDP-[^{14}C]glucose	96	<0.02
GDP-[^{14}C]glucose	60	<0.02
TDP-[^{14}C]glucose	158	<0.02
CDP-[^{14}C]glucose	30	<0.02
[^{14}C]Glucose-1-P	102	<0.02
[^{14}C]Glucose-1-P + 10^{-3} M 5′ AMP	102	<0.02

[a] The specific activities of the sugar nucleotides ranged from 6 to 14 × 10^5 cpm/μmole. The assay conditions were the same as described in assay A with the exception that 500 μg of soluble corn amylose was used.

Figure 4 shows that the rate of transfer of glucose from ADP-glucose is linear with increasing concentration of maltotriose and with maltose. Of all the oligosaccharides tested, only those of the maltodextrin series could act as acceptors (Table IV). Isomaltose, isomaltotriose, sucrose, cellobiose, gentiobiose, sophorose, laminaribiose, and glucose were all inactive as acceptor.

Identification of the Product. The formation of an α-1→4-glucosidic linkage by the transfer of the glucose from ADP-glucose to the primer was shown by the fact that when the radioactive alcohol-insoluble product was treated with α- or β-amylase, a 75% alcohol-soluble product was obtained. When the β-amylase-

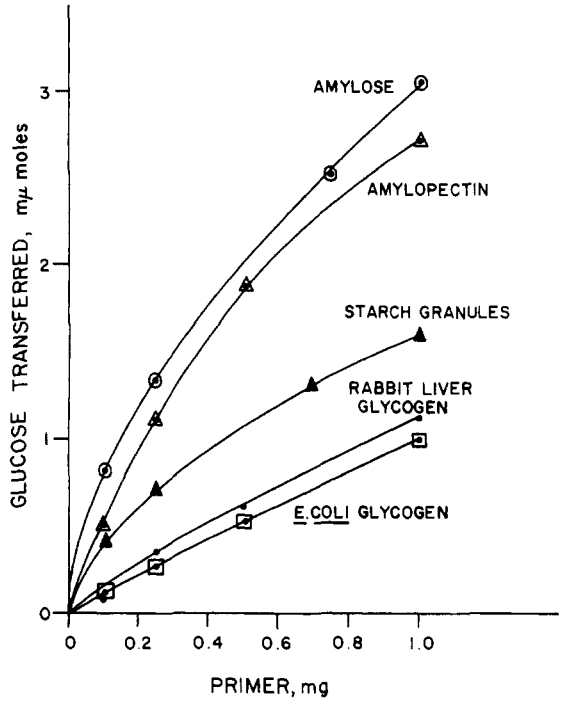

FIGURE 3: The incorporation of glucose from ADP-glucose into different α-1,4-glucans. Experimental conditions were identical to those described in the text.

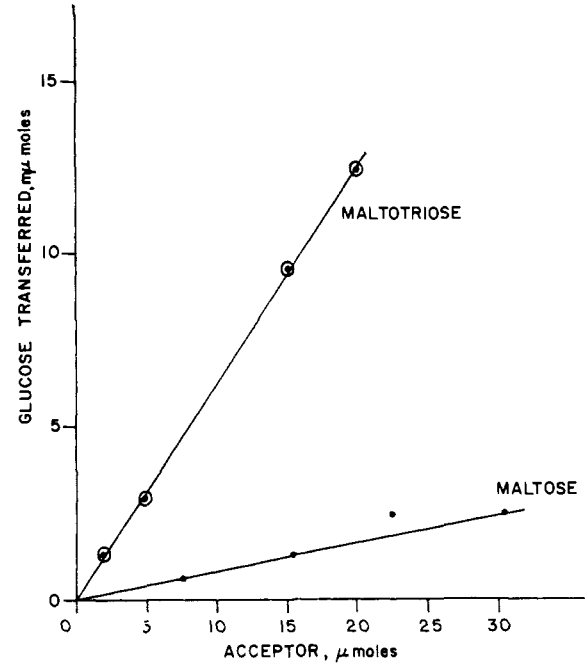

FIGURE 4: Dependence of the reaction rate on the oligosaccharide concentration. Experimental conditions are described in the text.

TABLE IV: Specificity of the Oligosaccharide Acceptors.[a]

Acceptor	Amount Present in Reaction Mixture (μmoles)	[^{14}C]Glucose Transferred (mμmoles)
Glucose	55	<0.02
Maltose	10	1.4
Isomaltose	3.0	<0.02
Maltotriose	2.0	1.8
Isomaltotriose	2.0	<0.02
Cellobiose	3.0	<0.02
Sucrose	3.0	<0.02
Gentiobiose	3.0	<0.02
Laminaribiose	3.0	<0.02
Sophorose	3.0	<0.02

[a] The conditions of the experiment are described in the text.

digested material was chromatographed in solvent systems B and C, all the radioactivity cochromatographed with maltose and had the same rate of migration as maltose in paper electrophoresis in 0.05 M borate buffer, *p*H 9.4. Hydrolysis of the radioactive transglucosylase product or its β-amylase digest with 1 N HCl for 2 hours gave a radioactive product which cochromatographed with glucose in three solvent systems (Table V).

Figure 5 shows that when maltose or maltotriose were used as acceptors in the transglucosylase reaction the radioactive products formed migrated on the paper as the corresponding higher oligosaccharides. Thus addition of maltose gave mainly [^{14}C]maltotriose and some [^{14}C]maltotetraose. Similarly, when maltotriose was used [^{14}C]maltotetraose was the main product together with small amounts of the higher oligosaccharides.

Properties of the Transglucosylase. The *p*H optimum of the transglucosylase was found to be 8.5 in both Tris-HCl and glycine-NaOH buffer (Figure 6). The activity of the transglucosylase was greater in the glycine-NaOH buffer.

Kinetics. The K_m for ADP-glucose was found to be about 1.8×10^{-4} M while the K_m for deoxy ADP-glucose was found to be about 4.5×10^{-4} M (Figure 2). Deoxy-ADP-glucose was also found to be a competitive inhibitor of ADP-glucose and its K_i was found to be 5.2×10^{-4} M.

Inhibitors and Activators. ADP was an inhibitor of the transglucosylase and appeared to be competitive with ADP-glucose; its K_i was 3.5×10^{-4} M (Figure 7). In the absence of glutathione, *p*-mercuribenzoate was found to be a potent inhibitor of the transglucosylase and the reaction was completely blocked by 5×10^{-5} M *p*-mercuribenzoate. Inhibition by even 10^{-3} M *p*-mercuribenzoate could, however, be counteracted by 10^{-2} M GSH. No activation or inhibition of transglucosylase was observed with glucose, glucose-1-P,

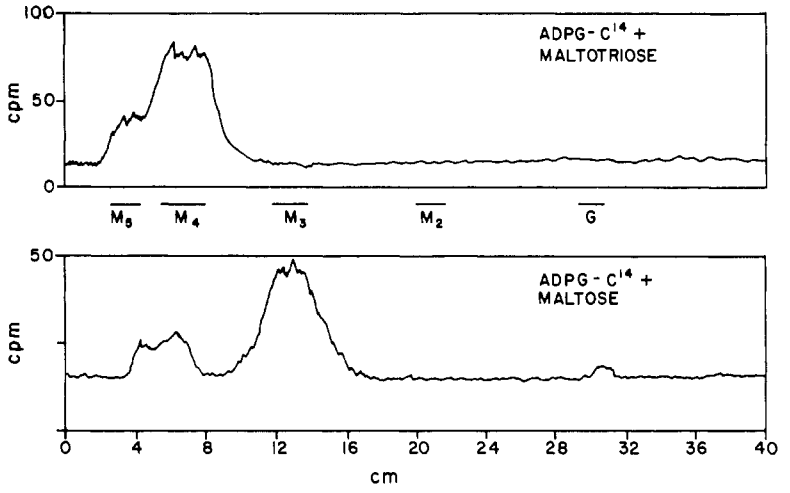

FIGURE 5: Transfer of glucose from ADP-glucose to maltose and maltotriose. The conditions of the experiment were those described for assay C. The filtrate from the Dowex 1 (Cl) treatment was concentrated and then chromatographed in solvent B for 46 hours. The papers were then counted in a Nuclear Chicago paper-strip counter. The abbreviations used are: G, glucose; M_2, maltose; M_3, maltotriose; M_4, maltotetraose; and M_5, maltopentaose.

TABLE V: Identification of the ^{14}C Products.[a]

Compound	R_F in Solvent System			Paper Electrophoresis
	A	B	C	
Glucose	1.00	1.00	1.00	20
Maltose		0.68	0.55	3.5
Maltotriose		0.45	0.32	2.2
Cellobiose		0.64	0.50	1.9
Mannose	1.25	1.15	1.14	13.5
Galactose	0.81	0.86	0.89	16.5
^{14}C product	0.0	0.0	0.0	
^{14}C product hydrolyzed in 1 N HCl	1.00	1.00	1.00	20.2
^{14}C product digested in the β-amylase		0.68	0.54	3.3

[a] The solvent systems are described in the text. The time of development was: solvent A, 16–24 hours; solvents B and C, 40–48 hours. The paper chromatographic data refer to the R_F relative to that of glucose. The electrophoretic data are expressed as cm migrated in 60 minutes in 0.05 M borate, *p*H 9.4, with a voltage gradient of 80 v/cm of paper

glucose-6-P, fructose-6-P, fructose 1,6-diphosphate, ribose-5-P, phosphoenolpyruvate, 3-phosphoglycerate, 2,3-diphosphoglycerate, pyruvate, and inorganic phosphate at a concentration of 10^{-3} M. Dihydroxyacetone phosphate was 35% inhibitory at 2.5×10^{-3} M while 3-phosphoglyceraldehyde was 97% inhibitory at 2.5×10^{-3} M. The inhibition by glyceraldehyde-3-P was found to be competitive in nature with ADP-glucose and a K_i of about 2.4×10^{-4} M was obtained. ATP was the only triphosphate found to be inhibitory (40%) when present at a concentration of about 2.5×10^{-3} M. AMP at a concentration of 2×10^{-3} M inhibited the transglycosylase by 40%. The enzymic activity was also inhibited to 60% by 2.5×10^{-3} M Mg^{2+}. Sugar nucleotides like UDP-glucose, TDP-glucose, GDP-glucose, CDP-glucose, ADP-galactose, ADP-mannose, UDP-galactose, GDP-mannose, TDP-galactose, and IDP-glucose at a concentration of about 5×10^{-4} had no appreciable effect on the transglucosylase.

Stoichiometry. Table VI shows that for every μmole of glucose transferred from ADP-glucose 1.05 μmoles of ADP is formed. Thus the reaction may be represented as ADP-glucose + α-1,4-glucan → α-1,4-glucosyl-glucan + ADP.

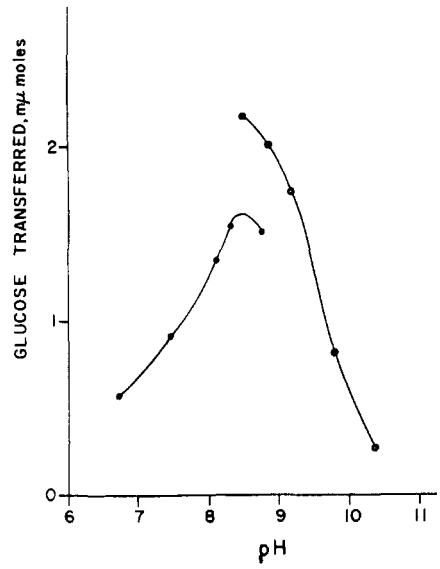

FIGURE 6: The variation in the transglucosylase activity with *p*H. The experimental conditions are the same as in assay A. The solid circles represent Tris-Cl buffer and the open circles represent glycine-NaOH buffer, respectively.

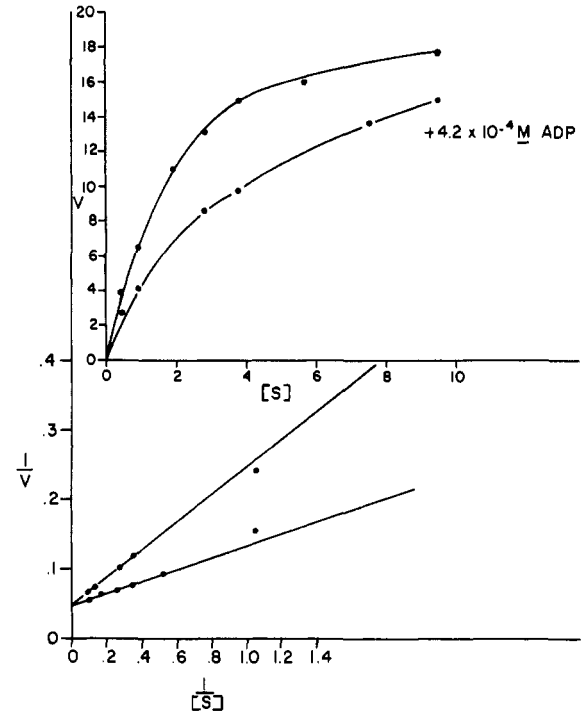

FIGURE 7: Competitive inhibition of the transglucosylase by ADP. Here V is mμmoles glucose incorporated in 15 minutes and S is in 10^{-4} M. The conditions of the experiment were the same as in assay A.

TABLE VI: Stoichiometry of the *Transglucosylase* Reaction.[a]

Time (min)	Glucose Incorporated (mμmoles)	ADP Formed (mμmoles)
0	0	7.0
30	123.0	137.0
Δ	123.0	130.0

[a] The reaction mixture contained 50 μmoles glycine-NaOH buffer, *p*H 8.5, 25 μmoles KCl, 10 μmoles GSH, 5 μmoles EDTA, 2.5 mg soluble corn amylose, 190 mμmoles of ADP-[^{14}C]glucose, and the purified enzyme in a total volume of 1 ml. After 30 minutes at 37°, the reaction was stopped by heating in boiling water for 30 seconds. A portion of the reaction mixture (0.1 ml) was taken for estimating the [^{14}C]glucose incorporated (assay A) and 0.7 ml of the mixture was analyzed for ADP formed (Kornberg and Pricer, 1950).

Discussion

Since the initial report of Leloir *et al.* (1961) describing the synthesis of α-1,4-glucosyl linkages in starch granules prepared from beans, many other starch granule systems transferring glucose from either ADP-glucose or UDP-glucose to starch have been reported (Recondo and Leloir, 1961; Akazawa *et al.*, 1964; Murata *et al.*, 1964; Murata and Akazawa, 1964). It has been only in the past year that soluble α-1,4-glucan transferases have been shown to be present in plants. The advantages of finding soluble transferases are obvious. They are more accessible to isolation and purification; their requirements for optimal catalysis can be studied as well as the specificity of their requirements. This is particularly true for the nature of the primer requirements for the soluble plant α-1,4-glucan transferases. The soluble preparation from sweet corn (Frydman and Cardini, 1964a) can use only amylopectin and glycogen as primers. Amylose, soluble starch, and starch granules were completely ineffective as primers. The soluble enzyme from tobacco leaves (Frydman and Cardini, 1964b) could use either amylopectin, glycogen, or heated starch granules as primer. The soluble enzyme from potato tubers (Frydman and Cardini, 1964b), however, could use only whole starch granules as the acceptor; amylopectin, glycogen, or heated starch granules were inactive as primers. Our results indicate that the requirement for primer of the spinach leaf chloroplast enzyme is similar to that of the tobacco leaf enzyme. Soluble amylose, amylopectin, heated starch granules, and glycogen, as well as malto-oligosaccharides, can act as primers.

The spinach chloroplast transglucosylase can utilize only ADP-glucose and deoxy-ADP-glucose as glucose donors; other glucose nucleotides tested were inactive. Sulfhydryl groups are essential for transglucosylase activity. It had been shown already that intact —SH groups were necessary for mammalian UDP-glucose–

glycogen transglucosylase (Kornfield and Brown, 1962) and for bacterial ADP-glucose–glycogen transglucosylase activity (Greenberg and Preiss, 1965). Unlike the mammalian or yeast transglucosylases (Leloir and Goldemberg, 1960; Algranati and Cabib, 1962), the chloroplast transglucosylase was not activated by glucose-6-P. In fact none of the glycolytic intermediates tested could stimulate the chloroplast transglucosylase activity. However it has been previously shown that the ADP-glucose pyrophosphorylase activity in spinach chloroplast is stimulated about 50-fold by 3-phosphoglyceric acid (Ghosh and Preiss, 1965). This finding might be of some significance to the control of starch formation during photosynthesis. In chloroplast extracts the level of ADP-glucose pyrophosphorylase is lower than the ADP-glucose–starch transglucosylase activity. Thus the synthesis of ADP-glucose probably is the limiting reaction in the synthesis of starch in chloroplasts. Therefore it is possible that during CO_2 fixation the accumulation of 3-phosphoglycerate causes an increase of synthesis of ADP-glucose by stimulation of the ADP-glucose pyrophosphorylase. An increase in the ADP-glucose concentration in the cell would then increase the rate of synthesis of starch. Regulation of starch synthesis would then be at the pyrophosphorylase level instead of at the transglucosylase level. This activation of the chloroplast ADP-glucose pyrophosphorylase by 3-phosphoglycerate is similar to the activations of the bacterial ADP-glucose pyrophosphorylases by glycolytic intermediates (Shen and Preiss, 1964, 1965a). It is possible that activation of both plant and bacterial α-1,4-glucan synthesis is at the pyrophosphorylase level. This would be in contrast to mammalian α-1,-4-glucan synthesis where activation occurs at the transglucosylase level (Craig and Larner, 1964; Rosell-Perez and Larner, 1964).

References

Akazawa, T., Minamikawa, T., and Murata, T. (1964), *Plant Physiol. 39*, 371.

Algranati, I. D., and Cabib, E. (1962), *J. Biol. Chem. 237*, 1007.

Anet, E. F. L. G., and Reynolds, T. M. (1954), *Nature 174*, 903.

Ashwell, G. (1957), *Methods Enzymol. 3*, 73.

Badenhuizen, N. P. (1963), *Nature 197*, 464.

Bray, G. (1960), *Anal. Biochem. 1*, 279.

Craig, J. W., and Larner, J. (1964), *Nature 202*, 971.

Doi, A., Doi, K., and Nikuni, Z. (1964), *Biochim. Biophys. Acta 92*, 628.

Frydman, R. B., and Cardini, C. E. (1964a), *Biochem. Biophys. Res. Commun. 14*, 353.

Frydman, R. B., and Cardini, C. E. (1964b), *Biochem. Biophys. Res. Commun. 17*, 407.

Ghosh, H. P., and Preiss, J. (1965), *J. Biol. Chem. 240*, PC 960.

Greenberg, E., and Preiss, J. (1965), *J. Biol. Chem. 240*, 2341.

Kochetkov, N. K., Budowsky, E. I., Shebaev, V. N., and Grochev, M. A. (1962), *Biochim. Biophys. Acta 59*, 747.

Kornberg, A., and Pricer, W. E. (1950), *J. Biol. Chem. 182*, 763.

Kornfield, R., and Brown, D. H. (1962), *J. Biol. Chem. 237*, 1772.

Leloir, L. F., De Fekete, M. A. R., and Cardini, C. E. (1961), *J. Biol. Chem. 236*, 636.

Leloir, L. F., and Goldemberg, S. H. (1960), *J. Biol. Chem. 235*, 919.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Murata, T., and Akazawa, T. (1964), *Biochem. Biophys. Res. Commun. 16*, 6.

Murata, T., Sugiyama, T., and Akazawa, T. (1964), *Arch. Biochem. Biophys. 107*, 92.

Murata, T., Sugiyama, T., and Akazawa, T. (1965), *Biochem. Biophys. Res. Commun. 18*, 371.

Recondo, E., and Leloir, L. F. (1961), *Biochem. Biophys. Res. Commun. 6*, 85.

Rosell-Perez, M., and Larner, J. (1964), *Biochemistry 3*, 773.

Roseman, S., Distler, J. J., Moffat, J. G., and Khorana, H. G. (1961), *J. Am. Chem. Soc. 83*, 659.

Schoch, T. J. (1957), *Methods Enzymol. 3*, 5.

Shen, L., and Preiss, J. (1964), *Biochem. Biophys. Res. Commun. 17*, 424.

Shen, L., and Preiss, J. (1965a), *Biochem. Biophys. Res. Commun. 18*, 180.

Shen, L., and Preiss, J. (1965b), *J. Biol. Chem. 240*, 2334.

Whatley, F. R., and Arnon, D. I. (1963), *Methods Enzymol. 6*, 308.