# MULTIPLE FORMS OF α-1,4 GLUCAN SYNTHETASE FROM SPINACH LEAVES[1]

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SUMMARY

ADP-glucose:α-1,4 glucan-4-glucosyl transferase was extracted from spinach leaves and separated by gradient elution on a DEAE-cellulose column. Fractions I and II gave equal activity with amylose, amylopectin, or glycogen as a primer while the activity of fraction III with glycogen as primer was only 25% of the activity observed with amylose or amylopectin as primer. Glucan synthesis in the absence of primer was found in fraction III. This activity was stimulated over 1000-fold by bovine plasma albumin and high salt concentrations. The radioactive unprimed product was a glucan with principally α-1,4 linkages and some α-1,6 linkages.

Both ADP-glucose:α-1,4 glucan-4-glucosyl transferase (1-4) and starch phosphorylase (5,6) have been implicated in the biosynthesis of the α-1,4 glucosidic linkages of starch. Of recent interest has been the problem of how synthesis of starch is initiated. Tsai and Nelson (5) have obtained evidence for the presence of multiple forms of phosphorylase in maize endosperm. Two of these forms are capable of synthesizing a polyglucoside in the absence of added α-1,4 glucan primer. They have proposed that phosphorylase may be involved in the "de novo" formation of starch. Slabnik and Frydman (6) have also found a phosphorylase in crude preparations from potato tubers that can catalyze polyglucose synthesis in the absence of primer. In both the above systems, high concentrations of glucose-1-P (15 to 20 mM) were used. Previous reports of unprimed polyglucose synthesis by highly purified phosphorylase have been discounted by the finding of small amounts of oligosaccharides either in

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the substrate glucose-1-P or as a contaminant of the enzyme preparation (7,8).

Conrad (9) has described ADP-glucose:α-1,4 glucan-4-glucosyl transferase in extracts of Aerobacter aerogenes which synthesizes glycogen without added primer. He suggests that the de novo process differs from the mechanism normally involved in glycogen biosynthesis.

The present communication reports the existence of multiple forms of ADP-glucose:α-1,4-glucan-4-glucosyl transferase in leaves of a higher plant (spinach), one of which can catalyze the formation of a polyglucose in the absence of added primer at a rate about two times faster than the primed reaction with physiological concentrations of ADP-glucose. Akazawa (personal communication) has found two isoenzymes of ADP-glucose:α-1,4 glucan-4-glucosyl transferase in extracts from rice endosperm.

## MATERIALS AND METHODS

Purification. Fresh deveined spinach leaves (150 g) from the local supermarket were washed and homogenized in a Waring blender with 200 ml of a solution containing 0.1 M phosphate buffer, pH 7.5, 0.01 M EDTA, and 0.005 M GSH for 2 minutes. All operations were carried out at 0-4°. The supernatant fraction resulting from centrifugation at 45,000 g for 20 minutes was made to 40% saturation with solid $(NH_4)_2SO_4$ and centrifuged at 30,000 g for 15 minutes. The precipitate was dissolved in about 20 ml of 0.05 M Hepes buffer, pH 7.0, containing 0.005 M dithiothreitol (DTT) and 0.01 M EDTA and dialyzed overnight against the same solution. The dialysate was placed on a DEAE-cellulose column (∿7 mg protein/ml of resin bed volume) which had been equilibrated with 15 resin bed volumes of 0.05 M Tris-acetate buffer, pH 8.5 containing 0.05 M EDTA, 0.002 M DTT, and 10% sucrose. After the passage of 1 column volume of the solution used for equilibration, 3 liters of the above Tris-acetate buffer with increasing KCl concentration (linear gradient 0-0.2 M KCl) was passed through the column and collected in 22.5 ml fractions. Appropriate fractions were combined and concentrated to 30 ml using an AMICON micropore ultrafiltrator with a P30 membrane and then further reduced to about 2 ml by precipitation with solid

$(NH_4)_2SO_4$ (40% saturation) and dialysed against 0.05 M Hepes buffer, pH 7.0, containing 0.005 M DTT, 0.01 M EDTA, and 10% sucrose.

Assay of transglucosylase. Incorporation of glucose into primer. The reaction mixture contained 140 mμmoles of ADP-[$^{14}$C]-glucose (500 cpm/mμmole), 20 μmoles of Bicine buffer, pH 8.5, 5 μmoles of potassium acetate, 2 μmoles GSH, 1 μmole of EDTA, 1 mg of amylopectin (amylose free) and enzyme in a final volume of 0.2 ml. After 15 or 30 minutes at 37°, the [$^{14}$C]-glucose incorporated into methanol-insoluble polysaccharide was determined (3).

Production of glucan without added primer. This assay was similar to the above assay except potassium acetate and amylopectin were replaced by 100 μmoles of Na citrate and 100 μg of bovine plasma albumin.

## RESULTS AND DISCUSSION

ADP-glucose:α-1,4 glucan-4-glucosyl transferase from spinach leaves was separated into three fractions by gradient elution from DEAE-cellulose. In

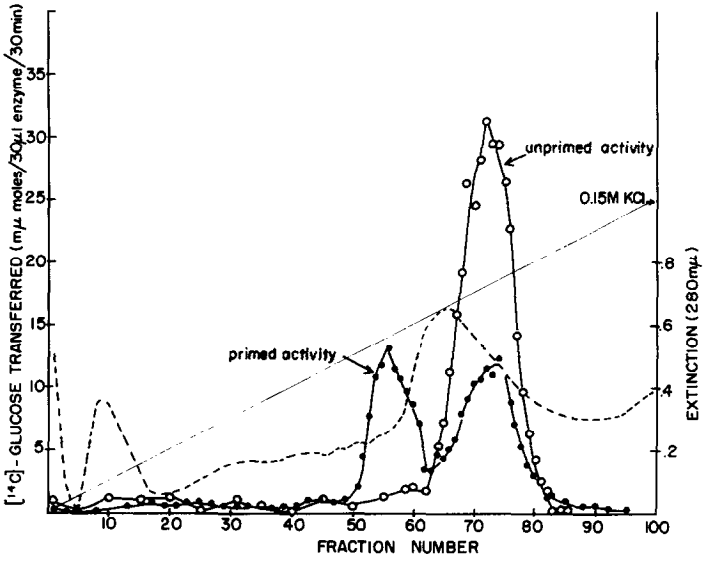


Fig. 1. Elution pattern of spinach leaf ADP-glucose:α-1,4 glucosyl transferase from DEAE-cellulose. Fractions were assayed with amylopectin (o) and without amylopectin in the presence of bovine plasma albumin and 0.5 M sodium citrate (o). Absorbance at 280 mμ is shown by the broken line.

[3]The pullulanase used was kindly provided to us by Dr. J. Robyt and Dr. D. French, Iowa State University of Science and Technology, Ames, Iowa 50010.

each preparation, 5% (or less) of the activity was recovered either in the wash through or in the first 100 ml of the gradient. The two other fractions with nearly equal activity were clearly separated (Fig. 1). The fractions will be referred to as transglucosylase I, II, and III in the order eluted from the DEAE-cellulose column.

Amylose, amylopectin, and glycogen were equally effective as primers for transglucosylase I and II, while activity of transglucosylase III with glycogen as primer gave only 25% of the activity observed with amylose or amylopectin as primer (Table I). Sucrose stabilizes the enzyme but does not serve as a primer.

Transglucosylase III catalyzed the synthesis of a methanol-insoluble glucose polymer in the absence of added primer (Fig. 1). In contrast to primed activity, the unprimed reaction was stimulated over 1000-fold by high concentrations of salts (e.g., 0.5 M Na citrate, 0.1 M EDTA, M NaF, M $(NH_4)_2SO_4$, or M potassium acetate, but not by M KCl, M KBr, M $NaClO_4$, or M KCNS). The addition of bovine plasma albumin gave a further stimulation resulting in a reaction rate twice the maximum rate obtained with high salts alone. The increase in activity resulting

TABLE I

INCORPORATION OF GLUCOSE FROM ADP-GLUCOSE

INTO DIFFERENT α-1,4 GLUCANS

One mg of primer was used in the reaction mixtures, details of which are given in the text. Reaction mixtures contained 100, 3 and 6.6 μg protein for Fractions I, II and III respectively.

| | [$^{14}$C]-Glucose Transferred (mμmoles/30 min) | | |
|---|---|---|---|
| Fraction | I | II | III |
| Acceptor | | | |
| Amylose (soluble corn) | 20 | 20 | 24 |
| Amylopectin (Calbiochem) | 20 | 20 | 28 |
| Glycogen | 24 | 24 | 6 |

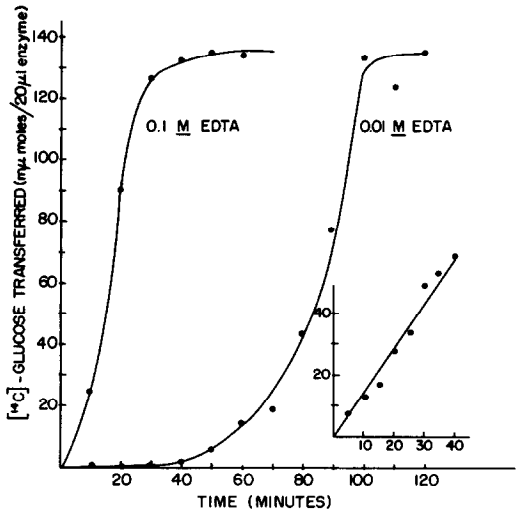


Fig. 2. Salt stimulation of unprimed ADP-glucose:α-1,4 glucosyl transferase. Enzyme activity was determined using 24 μg of spinach protein in the presence of 100 μg of bovine plasma albumin with 0.01 and 0.1 M sodium EDTA. Activity shown in the insert was measured using 6 μg of spinach protein in the presence of 100 μg bovine plasma albumin and 0.5 M sodium citrate. In each case the reaction mixture contained ADP [$^{14}$C] glucose bicine buffer, pH 8.5, and GSH as described in Materials and Methods.

from high salt concentrations and bovine plasma albumin was a consequence of shortening the lag phase of the unprimed reaction (Fig. 2). As the concentration of EDTA in the reaction mixture was increased from 0.01 M to 0.1 M, the lag time was shortened from over 70 minutes to less than 10 minutes. At higher salt concentration (e.g., 0.5 M Na citrate), the reaction was linear with time (see insert, Fig. 2).

Hydrolysis of the DEAE cellulose preparation of transglucosylase III with 2N HCl released 1 μg of glucose per mg of protein. Although it is possible that the hydrolyzable material associated with the transglucosylase is acting as a primer, the different conditions and faster rate of the unprimed reaction suggests that either a bound primer is present which is much more effective than added primer or that the reaction is truly de novo.

The radioactive product formed in the unprimed reaction was precipitated by methanol-KCl and remained at the origin during paper chromatography using 95% ethanol:M ammonium acetate, pH 3.8 (5:2 by vol) as developing solvent.

Most of the product (60-80%) was converted to maltose by β-amylase and nearly all of the product (95%) was converted to maltose and glucose by α-amylase. In the presence of pullulanase (10) and β-amylase the product was (97%) converted to maltose. The results are consistent with the product being a glucan containing principally α-1,4 linkages with some α-1,6 linkages.

These data suggest that there are at least two isoenzymes of soluble ADP-glucose:α-1,4 glucan-4-glucosyl transferase in spinach leaves, one of which can catalyze de novo glucan synthesis. Product formed by the de novo reaction may serve as a primer for other enzymes.

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