# ALLOSTERIC REGULATION OF URIDINE DIPHOSPHOGLUCOSE: D-FRUCTOSE-6-PHOSPHATE-2-GLUCOSYL TRANSFERASE (E.C.2.4.1.14)*

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Summary: Kinetic studies of wheat germ sucrose-P synthetase indicate that both substrates, fructose-6-P and UDP-glucose, exhibit sigmoidal saturation curves in the presence or absence of 22 mM $MgCl_2$. $MgCl_2$ stimulates the maximal velocity about 2-fold and decreases the apparent affinity of UDP-glucose, 3-fold. In view of these data, sucrose-P synthetase may be considered an allosteric enzyme involved in regulating the biosynthesis of sucrose.

Biosynthesis of sucrose may occur by two different pathways (1-5). It may be formed by transfer of glucose from UDP-glucose to fructose (reaction 1; UDP-glucose:D-fructose-2-glucosyl transferase, E.C.2.4.1.13) or by transfer of glucose from UDP-glucose to fructose-6-P to yield sucrose-P (reaction 2, sucrose-P synthetase) which is then hydrolyzed to sucrose by phosphatase action (6,7) (reaction 3).

(1) UDP-glucose + fructose $\longrightarrow$ sucrose + UDP

(2) UDP-glucose + fructose-6-P $\longrightarrow$ sucrose-P + UDP

(3) sucrose-P $\longrightarrow$ $P_i$ + sucrose

Both sucrose and starch are considered to be the major reserve carbon sources in various tissues of higher plants. A number of studies from our laboratory suggested strongly that the regulation of the biosynthesis of starch, either in leaves (8-10) or in endosperm tissue (11), is manifested by controlling ADP-glucose synthesis. The mechanism of regulation proposed (8-10) was based on the observations that photosynthetic intermediates such as 3-phosphoglycerate and fructose-6-P activated and inorganic phosphate inhibited ADP-glucose pyrophosphorylase activity (reaction 4).

ATP + α-glucose-1-P $\rightleftharpoons$ ADP-glucose + $PP_i$

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Since starch synthesis appeared to be regulated both in the leaf and reserve tissue, it was suspected that the enzymes involved in sucrose biosynthesis may also exhibit allosteric kinetic phenomena. A study of the kinetics of the wheat germ sucrose-P synthetase has indicated this to be the case.

## MATERIAL AND METHODS

1,5-Anhydroglucitol-6-phosphate was obtained from Dr. R. K. Crane of the University of Chicago. All other compounds were obtained from commercial sources in the highest purity available.

Assay A. Estimation of Sucrose-P Synthetase Activity. Sucrose-P synthetase activity was measured by determining formation of UDP dependent on the presence of fructose-6-P in the reaction mixture. The reaction mixture which contained in a volume of 0.15 ml, 0.5 µmole of UDP-glucose, 2.0 µmoles of fructose-6-P, 3.3 µmoles of $MgCl_2$, 150 µg of bovine serum albumin, 10 µmoles of Hepes (N-2-hydroxy-ethyl-piperazine-$N^1$-2-ethane-sulfonic acid) buffer, pH 6.5, and enzyme was incubated 15 minutes at 37°. After incubation, the reaction was terminated by heating for one minute in a boiling water bath. Water, 0.85 ml, was added to the heated mixture and after mixing, aliquots of either 0.3 ml or 0.5 ml were used for the determination of UDP with lactate dehydrogenase, pyruvate kinase, phosphoenolpyruvate, and DPNH. Oxidation of DPNH was measured spectrophotometrically at 340 mµ. No UDP was formed in the absence of either fructose-6-P, UDP-glucose or enzyme.

Assay B. Sucrose-P synthetase activity was also measured by the Roe method (12) as described by Cardini *et al.* (1). The incubation mixture was the same as described in Assay A. In Assay B the reaction was terminated by adding 0.02 ml of 5 N NaOH plus 0.33 ml of $H_2O$ and heating for 10 minutes in a boiling $H_2O$ bath. Assay A and Assay B gave essentially the same results when used to analyze sucrose-6-P synthetase activity.

Purification of the Wheat Germ Sucrose-6-P Synthetase. The enzyme was purified according to the procedure of Mendicino (3) and catalyzed the formation of 21.5 µmoles of UDP per mg of protein in 15 minutes. Sucrose synthetase

activity (reaction 1) was only 2% the rate of sucrose-P synthetase activity in this preparation. Kinetic studies were done under conditions where velocity was linear with both time and enzyme.

## RESULTS

Figure one shows that the saturation curve for fructose-6-P is sigmoidal in shape in the presence or absence of $MgCl_2$. A plot of the data according to the Hill equation (13) gives a value for $\bar{n}$, the apparent order of reaction for fructose-6-P, of 1.8 in the presence of $MgCl_2$ and 1.4 in the absence of $MgCl_2$.

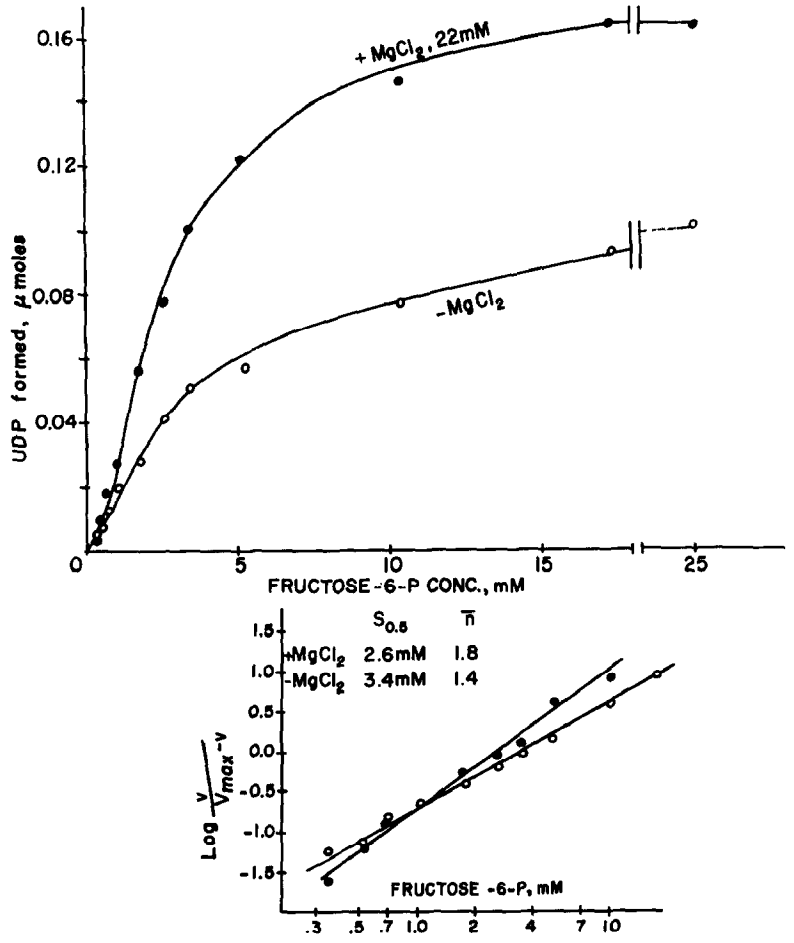


Fig. 1. The effect of fructose-6-P concentration on sucrose-P synthetase activity. The reaction mixtures and conditions of the experiment were the same as indicated in the text except $MgCl_2$ and fructose-6-P were varied as indicated. The amount of enzyme added to the reaction mixtures was 8.9 μg of protein when $MgCl_2$ was present and 19 μg of protein when $MgCl_2$ was absent.

There is only a slight decrease in the concentration of fructose-6-P required for half-maximal activity. However $Mg^{++}$ does significantly decrease the concentration of UDP-glucose needed for half maximal activity (Fig. 2). Once again the substrate saturation curve is sigmoidal whether $Mg^{++}$ is present or not. In addition to lowering the $S_{0.5}$ value for UDP-glucose, $Mg^{++}$ increases the maximal velocity of the reaction about 2-fold.

No effect of 1 mM 3-phosphoglycerate, fructose diP, $TPN^+$, $DPN^+$, DPNH, DPN, ADP, or UDP on the velocity of sucrose-P formation has been observed whether the substrates are present in the reaction mixture in saturating or less than saturating concentrations. However it was found that certain analogues of fructose-6-P could increase the rate of sucrose-6-P formation at unsaturating

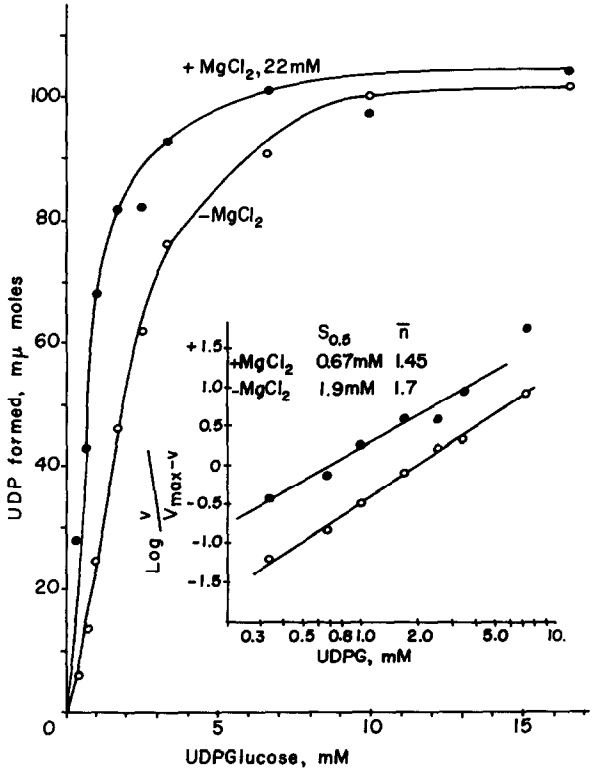


Fig. 2. The effect of UDP-glucose concentration on sucrose-P synthetase activity. The reaction mixtures and conditions of the experiment were the same as indicated in the text except $MgCl_2$ and UDP-glucose were varied as indicated. For reaction mixtures containing $MgCl_2$ 4.5 μg of protein were added and for reaction mixtures containing no $MgCl_2$, 9.5 μg of protein were added.

TABLE I

Stimulation of Sucrose-6-P Synthetase Activity
by 2-Deoxyglucose-6-P and 1,5-Anhydroglucitol-6-P

The reaction mixtures and conditions of the experiment were the same as described in the text. The fructose-6-P analogues (2-deoxyglucose-6-P and 1,5-anhydroglucitol-6-P) were added as indicated.

| Fructose-6-P Concentration | Sucrose-6-P Formed | | Stimulation |
|---|---|---|---|
| mM | mμmoles | | -fold |
| | | + 2-deoxyglucose 6-P, 13.3 mM | |
| 0 | 0.0 | 0.0 | - |
| 0.30 | 3.2 | 20 | 6.3 |
| 0.46 | 9.7 | 29 | 3.0 |
| 0.76 | 20 | 48 | 2.4 |
| 1.52 | 45 | 99 | 2.2 |
| 3.04 | 94 | 129 | 1.4 |
| 12.2 | 146 | 149 | 1.0 |
| | | + 1,5-anhydroglucitol 6-P, 13.3 mM | |
| 0 | 0.0 | 0.0 | - |
| 0.46 | 5.6 | 22 | 3.9 |
| 0.92 | 15 | 40 | 2.7 |
| 1.52 | 22 | 53 | 2.4 |
| 3.04 | 49 | 70 | 1.4 |
| 12.2 | 72 | 75 | 1.0 |

concentrations of fructose-6-P (Table I). At 0.46 mM fructose-6-P the stimulation observed in the presence of 2-deoxyglucose-6-P (13.3 mM) and 1,5-anhydroglucitol-6-P (13.3 mM) was 3.0- and 3.9-fold, respectively. These fructose 6-P

analogues however cannot substitute for fructose-6-P in the sucrose-6-P synthetase reaction.

## DISCUSSION

The above data indicate that the substrates for sucrose-P synthetase reaction exhibits sigmoidal shaped saturation curves similar to those observed for many allosteric enzymes. These type of kinetic data may suggest multiple and interacting binding sites for the substrates and in the case of fructose-6-P the stimulation of activity by the analogues is consistent with this concept. Whereas both 2-deoxyglucose-6-P and 1,5-anhydroglucitol-6-P cannot bind to the fructose-6-P substrate site they appear to replace fructose-6-P at the activator site(s).

Sucrose-6-P synthetase activity is therefore modulated by the concentration of the substrates, UDP-glucose and fructose-6-P. The sigmoidal shape of the substrate saturation curves would provide a more sensitive response to the fluctuation of substrate concentrations. The properties of the synthetase as seen *in vitro* suggest that sucrose synthesis in wheat germ may be regulated at the level of sucrose-6-P synthesis. Although these results have been obtained with a sucrose-6-P synthetase from non-photosynthetic tissue, it is tempting to speculate that the same allosteric properties may be associated with the chloroplast sucrose-6-P synthetase (14). The rapid rate of sucrose synthesis in chloroplasts during photosynthesis could then be explained by the increase of both UDP-glucose and fructose-6-P due to $CO_2$ fixation (15-17). It should be pointed out that previous studies on sucrose-P synthetase did not reveal the sigmoidal nature of the substrate curves (2,3). However concentrations lower than 0.8 mM for either fructose-6-P or for UDP-glucose were not employed in those studies. The sigmoidicity of the substrate curves would thus be missed.

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