Enzymic Mechanism of Starch Synthesis in Plants

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An important development in the field of polysaccharide biochemistry has been the discovery of starch synthetase in beans, potatoes and corn seedlings by Leloir and coworkers[1].

The enzyme was found to be closely bound to the starch granules and to catalyze the transfer of glucose moiety from UDP-glucose into polysaccharides containing linkage of the α-D-(1→4) type.

Subsequently, the granule-bound starch synthetase was observed to transfer the glucose residues into starch being 10 times as fast from ADP-glucose as from UDP-glucose[2].

The author et al. have also found the similar enzyme in some plants, including rice grains[3),4)] and soybean leaves[5] as well as a soluble starch synthetase in glutinous rice grains[6], where the starch granule-bound enzyme is not detectable.

At present it may be said that the accumulation of many data has established a basic enzymic mechanism of the starch synthesis. It is not, however, still known about a relationship between the physical structure of starch granules and its biosynthetic process and about the enzymic mechanism for the formation of α-D-(1→6) bonds (branching points) in starch molecules.

I have recently found that the granule-bound starch synthetase of various plant origins requires K^+ for its maximum activity[7),8)] and that sucrose synthetase has a regulatory property in the reaction of sucrose cleavage[9),10)]. This paper deals with these experiments.

Mechanism of glucose transfer from sucrose into starch

In some plants, including sweet potato and rice, sucrose is synthesized in the leaves and stems, and is translocated to the roots or grains where it eventually is transformed into starch. An enzymic mechanism of glucose transfer from sucrose into the starch molecules via ADP-glucose has been revealed by the works of an Argentine group and the author et al.

Fig. 1 is a sequence of the reactions of sucrose-starch conversion postulated by De Fekete and Cardini[11]. Similar results were

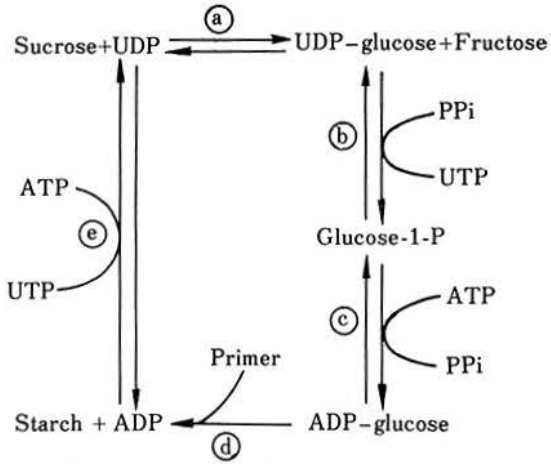


Fig. 1. Mechanism of glucose transfer from sucrose to starch (from De Fekete and Cardini[11])

obtained by the author et al.[4),12)] with enzyme systems of ripening rice grains. When ADP-glucose pyrophosphorylase was coupled with ADP-glucose-starch synthetase, glucose was transferred into starch molecules. The same result was obtained with ADP-sucrose synthetase. The corresponding UDP-glucose systems were insufficient in promoting the transfer of glucose.

The major objection to the reactions presented in Fig. 1 lies in the fact that the D-glucose-1-phosphate (G1P) formation from UDP-glucose by the pyrophosphorylase reaction is physiologically quite unfavorable. As has been emphasized by Kornberg, enzymic reactions releasing pyrophosphate (pp_i) are considered to be essentially irreversible due to the hydrolysis of pp_i, thus making the overall reaction a synthetic one. Therefore, I have been extensively looking for another mechanism of G1P formation than the pyrophosphorylase reaction.

Stimulative effect of K^+ on starch synthetase

Some classical works have led to circumstantial evidences indicating the close connection between K^+ and starch synthesis in plant cells. As yet no single enzyme involved in the polysaccharide synthesis had been shown explicity to require K^+. Since it has long been known that the application of potassium fertilizer is effective for the growth of its roots, I examined an effect of K^+ on the granule-bound starch synthetase of sweet potato roots prepared by the thorough washing with glass-pot distilled water.

As shown in Fig. 2, the very low glucose incorporation into starch from ADP-glucose was observed without K^+, while the addition of K^+ in the reaction mixtures markedly stimulated the enzyme activity about 7 fold. The flame photometric analysis has shown the presence of a minute amount of K^+ attached to the starch granules. Thus, the activity seen without the addition of K^+ is assumed to be due to K^+ endogenous to the granules.

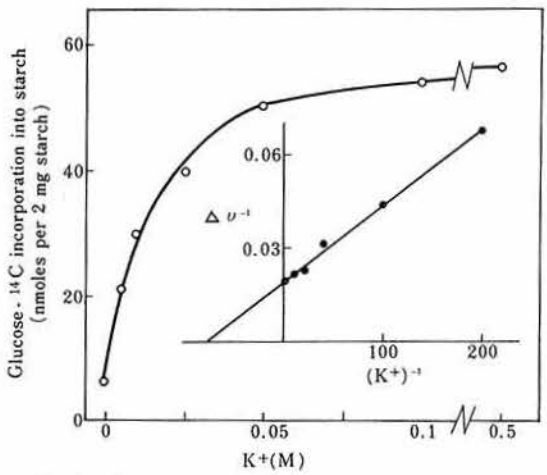


Fig. 2. Stimulative effect of various concentration of K^+ on the activity of ADP-glucose starch synthetase of sweet potato roots. Incubation was at 34°C for 60 min. with reaction mixtures containing 154 nmoles of ADP-glucose-^{14}C (13,420 dpm)

The level of K^+ giving maximum reaction velocity was about 0.05–0.1 M, which was roughly equivalent to the cellular concentration of K^+ in the sweet potato roots.

From the kinetic analysis of K^+ concentration vs. the rate of the starch synthetase reaction (inset of Fig. 2), the following values were obtained: K_A for $K^+=1.33\times10^{-2}$ M, and $V_{max}=27.1$ nmoles glucose incorporated into starch per hour per mg starch granules.

Experimental results concerning the effect of univalent cations on the activity of starch synthetase of sweet potato roots show that the most pronounced stimulative effect is given by K^+, while Rb^+, Cs^+ and NH_4^+ exhibited also a marked stimulation. However, neither Na^+ nor Li^+ as well as anions exhibit a great stimulation on the enzyme reaction.

I next examined the stimulative effect of K^+ on the starch synthetase of various plant origins. Results in Table 1 show that the stimulative effect of K^+ was most marked with sweet potato enzyme. It will be noted that promotion of enzyme activity was uniformly observed with preparations from all three tissues of sweet potato, although the specific enzyme activities (nmoles glucose transferred

Table 1. Effect of K^+ on granule-bound ADP-glucose starch synthetase activity of various plant origins

Source of enzyme	KCl (0.1M)	Glucose transferred (nmoles/mg starch)	Relative activity (%)
Sweet potato roots	—	7.6	100
	+	52.5	691
Sweet potato leaves	—	20.6	100
	+	147.2	714
Sweet potato stems	—	15.4	100
	+	108.5	705
Potato tubers	—	34.3	100
	+	65.5	191
Rice grains	—	18.8	100
	+	25.6	136
Barley seeds	—	26.7	100
	+	36.2	135
Broad bean seeds	—	7.9	100
	+	27.3	348
Taro tubers	—	6.7	100
	+	8.4	125
Konjak bulbs	—	23.5	100
	+	39.1	167

Reactions were carried out at 37°C for 60 min. with reaction mixtures containing 285 nmoles of ADP-glucose

per mg starch granules) of the leaf and stem preparations were 3- and 2-fold higher than that of root preparation, respectively.

Broad bean enzyme was also appreciably stimulated by K^+. It is noteworthy that broad bean seeds contain a relatively high content of K^+ of 1.35% (dry weight basis), which is roughly equivalent to 0.06 M. This value was a little lower than that in sweet potato roots but much higher than that in rice grains (ca. 0.3%).

Although about 2-fold activation due to K^+ addition occurred for the potato tuber enzyme, maximum activity with K^+ addition was rather higher than that for sweet potato root enzyme. Experimental results concerning the effect of various concentration of K^+ on the activity of potato tuber enzyme showed that the level of K^+ giving the maximum effect was around 0.02 M.

In rice grain enzyme, on the other hand, the optimum K^+ level was at 0.01–0.02 M, which is equivalent to the K^+ content in rice grains and higher K^+ concentration caused a somewhat inhibitory effect.

Potassium ion had relatively little effect on the activity of enzyme preparations from barley, taro and konjak. It is interesting that greater stimulation by K^+ was observed in the starch synthetase from tissues of the plant such as sweet potato and broad bean which show higher potassium content.

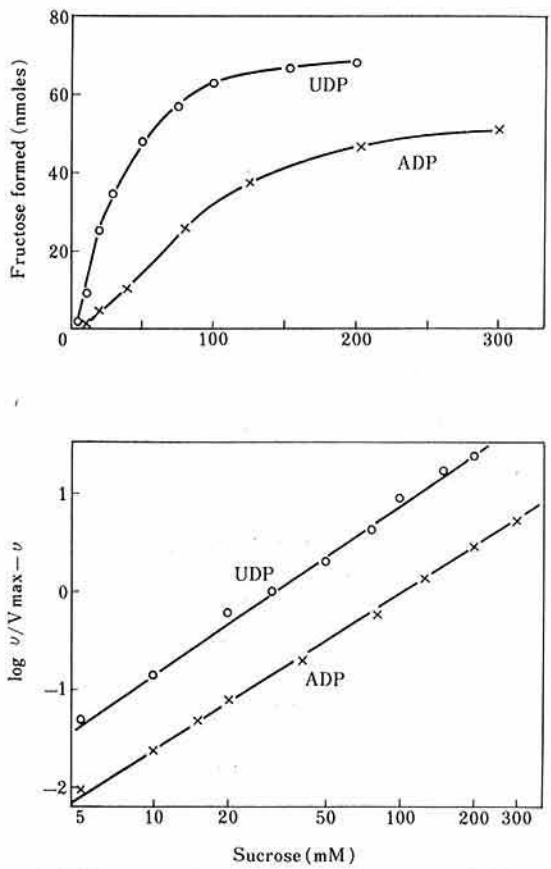
Regulatory property of sucrose synthetase

Recent works on sucrose synthetase isolated from various plant tissues indicate that a crucial role of the enzyme is a metabolic bridge between polysaccharide formation as an example shown in Fig. 1.

In sweet potato, the content of both sucrose and UDP-glucose is relatively high at the initial development stage of root tissues and that at the actively developing stage the sucrose content remains rather constant, at about 0.7–1% of fresh weight[13].

It was thus surmised that the sucrose cleavage reaction in sweet potato roots may be regulated at the level of sucrose synthetase reaction. In order to examine this hypothetical view, some kinetic properties were studied with purified sucrose synthetase from sweet potato roots and others.

Results of Fig. 3 show the effect of sucrose concentration on the sucrose cleavage reaction by the sucrose synthetase in the presence of either UDP or ADP as a glucose acceptor. Both saturation curves for sucrose are deviated from the Michaelis-Menten equation (1) and are sigmoidal in shape. It can be seen that the reaction rate is accelerated at about 10–15 mM and 30–40 mM sucrose in the presence of UDP and ADP, respectively. By plotting the data following the Hill equation (2), straight lines were obtained and the interaction coefficient (n value) and the sucrose concentration required for half



○ UDP-sucrose cleavage activity, enzyme protein 0.24 μg
× ADP-sucrose cleavage activity, enzyme protein 0.96 μg

Fig. 3. Effect of sucrose concentration on the activity of sucrose synthetase in sucrose cleavage reaction

$$\frac{1}{v}=\frac{Km}{V_{max}}\cdot\frac{1}{(S)}+\frac{1}{V_{max}} \qquad (1)$$

$$\log\frac{v}{V_{max}-v}=n\log(S)-\log K \qquad (2)$$

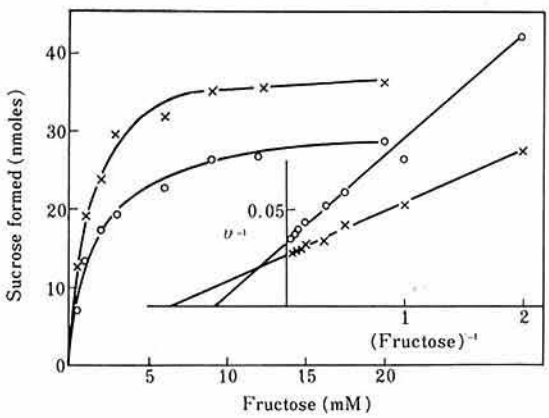
v : Reaction velocity
(S) : Substrate concentration
V_{max}: Maximum velocity at saturated concentration of substrate
Km : Michaelis constant

maximal activity ($S_{0.5}$) were calculated as follows: for UDP-sucrose cleavage reaction, n=1.7, $S_{0.5}$=31 mM sucrose and for ADP-sucrose cleavage reaction, n=1.6 and $S_{0.5}$=125 mM sucrose.

The enzyme characterized by the sigmoidal substrate saturation curve and by having n value greater than 1.0 is designated allosteric enzyme by Monod et al. Therefore, the kinetic data described above may strongly suggest the regulatory function of sucrose synthetase in the breakdown of sucrose molecules in sweet potato roots.

In this context, it is interesting to note that the content of sucrose in sweet potato roots is 30–45 mmoles per kg fresh weight at the initial developing stage, while it is 20–30 mmoles per kg at the actively developing stage when active photosynthesis proceeds.

In the UDP-sucrose cleavage reaction, the content of sucrose in sweet potato roots at these levels is approximately equal to the sucrose concentration of $S_{0.5}$. In contrast, for the ADP-sucrose system, the content of sucrose is quite comparable or even smaller than the threshhold concentration of sucrose, at which the reaction rate begins to increase. These results are quite unfavorable for the formation of ADP-glucose by ADP-sucrose cleavage reaction, which is also well known to be strongly inhibited by uridine nucleotides in vitro[11),12)].



○ No $MnCl_2$ added, enzyme protein 1.3 μg
× 5 mM $MnCl_2$ added, enzyme protein 0.9 μg

Fig. 4. Effect of fructose concentration on the activity of sucrose synthetase in UDP-glucose-sucrose synthesizing reaction

Table 2. Kinetic data of sucrose synthetase from various plant tissues

	Sucrose synthesis reaction				Sucrose cleavage reaction					
1st substrate	UDP-glucose		Fructose		Sucrose		Sucrose		UDP	
2nd substrate	Fructose		UDP-glucose		UDP		ADP		Sucrose	
Enzyme source	Km (mM)	n	Km (mM)	n	$S_{0.5}$ (mM)	n	$S_{0.5}$ (mM)	n	$S_{0.5}$ (mM)	n
Sweet potato roots	1.7	1.0	1.5	1.0	31	1.7	125	1.6	0.13	1.8
Potato tubers	2.0	1.0	1.4	1.0	55	1.5	183	1.4	0.14	1.8
Rice grains	2.7	1.0	3.3	1.0	30	1.6	105	1.6	0.11	1.8

The rate of sucrose synthesizing reaction as a function of fructose concentration is presented in Fig. 4, showing hyperbolic saturation curves for both in the presence and absence of $MnCl_2$ which is an activator of the reaction.

The reactions obeyed Michaelis-Menten equation (inset of Fig.) and gave straight lines with a slope (n value) of 1.0 by plotting the data according to Hill equation. The saturation curve for UDP-glucose was also hyperbolic in shape with an n value of 1.0. These results mean that the sucrose synthetase has no function of metabolic regulation in sucrose synthesizing reaction in contrast to sucrose cleavage one.

I have next examined whether or not the kinetic properties of sucrose synthetase isolated from other plant sources show similar behaviors observed with sweet potato root enzyme.

The data summarized in Table 2 indicate that the reaction kinetics obtained with the sucrose synthetase of both rice grains and potato tubers are basically similar to those of the sweet potato enzyme. Such an analogous behavior of the enzyme with respect to its substrate concentrations provides further evidence to a view that the enzyme breakdown of sucrose may be sensitively modulated by the reversal of the sucrose synthetase reaction.

In this connection, it is interesting to recall that Preiss and Greenberg[14] and the author[15] have reported that sucrose-phosphate synthetase of various plant origins is under allosteric control and the biosynthesis of sucrose is regulated at the level of the synthesis of sucrose-phosphate. Therefore, it is quite reasonable to think that the physiological role of sucrose synthetase would be to catalyze sucrose cleavage reaction but not sucrose synthesis reaction in plant cells.

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