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Some Properties of Potato Tuber UDPG:D-fructose-2-glucosyltransferase (E.C. 2.4.1.14) and UDPG:D-fructose-6-phosphate-2-glucosyltransferase (E.C. 2.4.1.13)[1]

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Abstract. **Sucrose and sucrose 6-phosphate synthetase were isolated from potato tubers, partially purified and their properties studied. The sucrose synthetase showed optimum activity at 45° and was inhibited competitively by ADP and some phenolic glucosides. The Ki's for these inhibitors were determined. Mg^{2+} was found to activate this enzyme. Activity toward UDP-glucose or ADP-glucose formation was measured. The optimum conditions for sucrose and UDP-glucose formation were found to differ. The specificity for the glucosyl donor and acceptor were determined.**

The optimum conditions for sucrose 6-phosphate synthetase activity were studied. This enzyme was not inhibited by either ADP or phenolic glucosides; UDP-glucose was the only glucosyl donor for sucrose 6-phosphate formation.

The synthesis of sucrose from a phosphorylated precursor, UDP-glucose was first suggested by Buchanan *et al.* (1). This suggestion was confirmed when Leloir *et al.* (2, 3) and others (4, 5, 6, 7) found that extracts from wheat germ, peas, sugar beet and other plants contain enzymes capable of catalyzing the synthesis of sucrose by transfer of glucose from UDP-glucose to either fructose or fructose 6-P.

The existence of 2 separate mechanisms for the synthesis of sucrose raised the question of their respective roles *in vivo*. Some results (8, 9, 10) suggest that sucrose 6-P is synthesized before sucrose, and is then hydrolyzed to the free disaccharide, indicating that this might be the mechanism of sucrose synthesis *in vivo*. The fact that sucrose 6-P synthetase is located in the chloroplasts (11) would also support this view. Sucrose synthetase probably does play an important role in the formation of the sugar nucleotides UDP-glucose and ADP-glucose (12, 13) although the equilibrium constant of the reaction favors sucrose synthesis (14). The 2 sucrose synthesizing enzymes were reported to be present in *Solanum tuberosum* tubers by Schwimmer *et al.* (15). Since these tubers are able to transform accumulated starch into sucrose in the cold, and *vice versa* at room temperature (16), it seemed that isolation and study of the properties of both synthetases might throw some light on their specific roles in the synthesis and transformations of sucrose.

Materials and Methods

Purine and pyrimidine nucleotides and UDP-D-glucose were purchased from Sigma Chemical Company, St. Louis, Missouri. ADP-D-glucose, GDP-D-glucose, and TDP-D-glucose were prepared synthetically according to the method of Khorana *et al.* (17) as slightly modified by Recondo (18). *p*-Methoxy-phenyl gentiobioside was prepared by the method of Nath and Ryden (19) and *p*-hydroxyphenyl cellobioside was a gift from Dr. S. Kocourek of Charles University, Prague. The other phenyl glycosides, chlorogenic and shikimic acids and solanine were commercial samples. The barium salt of fructose 6-P was transformed into the sodium salt by addition of $Na_2(SO_4)$ in an acidified medium.

Chromatography was carried out on Whatman No. 4 paper; solvent used: butyl alcohol-pyridine-water (6:4:3). The spots were located with alkaline silver nitrate (20). Protein was determined by the method of Lowry (21).

Assay of Sucrose or Sucrose 6-Phosphate Synthetase. The reaction mixture, which contained, in a final volume of 35 μl, 0.5 μmole of fructose or fructose 6-P, 0.5 μmole of tris maleate buffer (pH 7.5), 0.1 μmole of UDP-D-glucose or other sugar nucleotides and enzyme (10 μl), was incubated for 30 minutes at 37°. The residual fructose or fructose 6-P was destroyed by the addition of 20 μl of 10 N NaOH and heating at 100° for 10 minutes.

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and the sucrose or sucrose 6-P formed was assayed by the thiobarbituric method (22). Higher buffer concentrations did not alter the results. Absorbancy was measured in a Beckman spectrophotometer at 432 mμ. When the reverse reaction was measured, sucrose-^{14}C was employed and the radioactive nucleotides ADP-glucose-^{14}C or UDP-glucose-^{14}C were identified by paper chromatography with ethanol-ammonium acetate (pH 7.2). The radioactivity was measured with a gas flow counter. The reverse reaction was also determined by measuring reducing sugars by the method of Somogyi (23).

Assay of Phosphatase. The reaction mixture, which contained 0.5 μmole of fructose 6-P; 0.5 μmole of tris-maleate buffer (pH 7.5) and enzymes (10 μl) in a total volume of 30 μl, was incubated for 30 minutes at 37°. The P_i released was assayed by the Fiske and Subbarow method (24).

Assay of Invertase. The reaction mixture contained, in a final volume of 35 μl, 0.05 μmole of sucrose, 0.5 μmole of tris-maleate buffer (pH 7.2) and enzyme (10–20 μl). The incubation was carried out at 37° for 30 minutes. Blanks were run in which sucrose or enzyme was added at the end of incubation. Sucrose was determined as described before.

Isolation of Sucrose and Sucrose 6-P Synthetase. Potatoes (Kennebec variety) were kindly provided by the Instituto Nacional de Tecnologia Agropecuaria (INTA). A typical preparation is described: Peeled potatoes (100–200 g) were sliced and left for 10 minutes in a 7 0/00 solution of hydrosulfate, rinsed with distilled water and grated on a glass grater. The suspension was filtered through cheesecloth and to this crude extract mercaptoethanol was added (5 mM final conc). The particulate matter was separated by centrifuging at 1000 × *g*, the supernatant was centrifuged again for 15 minutes at 20,000 × *g* and the supernatant liquid was used as the enzyme source. Purification procedure: The 20,000 × *g* supernatant liquid was fractionated between 0 to 30, 30 to 40 and 40 to 50 % saturation with solid ammonium sulfate. The precipitates were dissolved in 0.01 M tris HCl buffer (pH 7.2) and dialyzed overnight against the same buffer containing 1 mM EDTA and 5 mM mercaptoethanol. The active fractions were then reprecipitated between 0 to 30 and 30 to 40 % ammonium sulfate saturation, the 0 to 30 % fraction being enriched in sucrose 6-P synthetase and the 30 to 40 % fraction almost free of this enzyme (table I). The last fraction was refractionated with a neutralized saturated ammonium sulfate solution into 2 fractions from 0 to 30 % (Fraction I) and from 30 to 40 % saturation (Fraction II). These preparations were free from phosphatase and invertase activity, and were purified about 3 times from the first ammonium sulfate precipitate.

When fraction II was passed through a DEAE cellulose column and eluted stepwise with 10 mM tris HCl buffer (pH 7.2) and NaCl, the enzyme was eluted between 0.15 and 0.25 M NaCl, but was absolutely unstable, thus making us unable to use this preparation which was about 20-fold purified from the 30 to 40 % ammonium sulfate precipitate.

Results

Properties of the Enzyme. Most of the sucrose 6-P synthetase activity precipitated between 0 to 30 % saturation with ammonium sulfate, and this was the fraction used for studying the enzyme (table I). For sucrose synthetase activity, fraction II of the 30 to 40 % ammonium sulfate preparation was used. The enzymes were quite unstable and lost about 40 % of their activity after 3 days when kept at 0 to 4°. No advantage was found in keeping the enzymes at −15°. After 15 days, almost no activity was left.

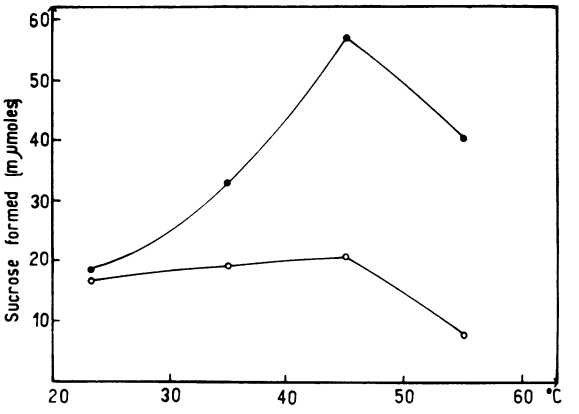

Fig. 1. Effect of temperature on the sucrose and sucrose-6-phosphate synthetases: The reaction conditions were as described in Methods, except for the temperature, which is varied as indicated in the abscissa. ● Sucrose synthetase, ○ Sucrose 6-P synthetase.

Table I. *Distribution of Sucrose and Sucrose 6-Phosphate Synthetase Activities*
The reaction conditions are described in Methods.

Ammonium sulfate fraction Saturation	Sucrose synthesizing activity Sucrose	Sucrose 6-P synthesizing activity Sucrose 6-P	Specific activity Sucrose per mg prot.	Specific activity Sucrose 6-P per mg prot.
%	*mμmoles*	*mμmoles*	*mμmoles*	*mμmoles*
0–30	37	34	2.1	2
30–40	39	6	2.4	0.3

Effect of Temperature. The activity of sucrose synthetase increased with temperature, showing a maximum at 45° (fig 1). At this temperature its activity was twice the activity at 23°. This enzyme seemed quite active at high temperatures up to 55°.

Sucrose 6-P synthetase, on the contrary, lost about 50 % of its activity at this temperature.

Effect of pH. Sucrose synthetase had maximum activity between a broad pH range, running from pH 7 to 9 with a decrease toward acid pH values (fig 2). The sucrose 6-P synthetase pH optimum went from 6.2 to 7.5 and decreased faster at alkaline pH values (fig 2).

Specificity of the Glucosyl Acceptor. In addition to fructose, sorbose and tagatose could also act as substrates for sucrose synthetase, although at a very low rate.

Specificity of the Glucosyl Donor. UDPglucose was found to be the best glucosyl donor for both enzymes, but, whereas ADPglucose seemed to be a quite good donor for sucrose synthetase, it was a very poor one for sucrose 6-P synthetase (fig 3). The K*m* and V*m* values for the different glucosyl donors of UDPglucose fructosyl transferase are shown in table II. The affinity of the enzyme for different nucleotides is quite similar (except for ADPglucose), although the maximum velocities differ markedly.

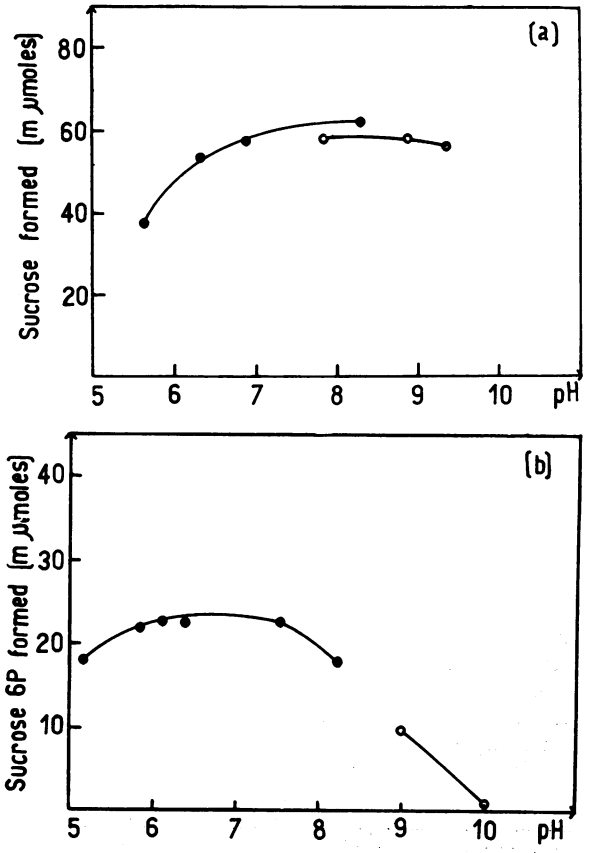

FIG. 2. Effect of pH on the rate of (a) sucrose, and (b) sucrose 6-P formation: The reaction mixture as described in Methods. ● Tris maleate buffer, ○ Glycine buffer.

Table II. *Michaelis Constants and Maximal Velocities of Sucrose Synthetase for Different Sugar Nucleotides*

The reaction mixture contained: fructose (0.5 µmole); tris maleate buffer (0.5 µmole) pH 7.5; 10 µmoles of sucrose synthetase, fraction II (20 mg of protein/ml) and 0.1 µmole of the indicated sugar nucleotides, were incubated at 37° for 30 minutes. The final volume was 35 µl. The sucrose formed was determined as described in Methods.

Glucosyl donor	K*m*	V max
		mµmoles per ml enzyme per hr
UDP-D-glucose	1.65×10^{-3} M	13,300
ADP-D-glucose	8.6×10^{-3} M	7500
TDP-D-glucose	1.0×10^{-3} M	3700
GDP-D-glucose	2.77×10^{-3} M	2600

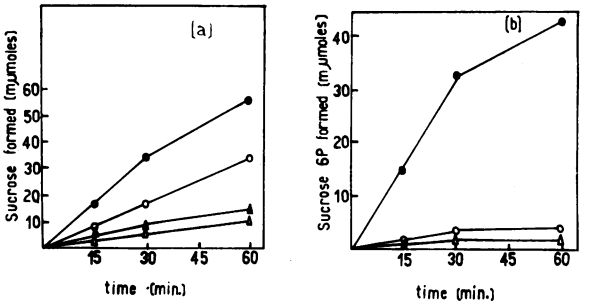

FIG. 3. Time course of sucrose and sucrose 6-phosphate synthesis from different sugar nucleotides: Conditions as described in Methods, except different times of incubation. The concentration of the sugar nucleotides were the same. ● UDP-glucose, ○ ADP-glucose, ▲ TDP-glucose, △ GDP-glucose.

Kinetics. The effect of substrate concentration on the rate of sucrose and sucrose 6-P synthesis is presented in figure 4. The apparent substrate enzyme affinity constants (K*m*) found for the different reactants were: for sucrose synthetase, UDPglucose 1.65 mM, fructose 5.9 mM and for sucrose 6-P synthetase, UDPglucose, 7.4 mM and fructose 6-P, 5.5 mM.

Reversibility of the Reaction. When the reaction catalyzed by sucrose synthetase was measured in the direction of synthesis of the sugar nucleotide, both UDP and ADP could act as glucose acceptors, but only when citrate buffer was used (table III). The fact that almost no reversibility was found with tris buffer (pH 7.5) under conditions which were optimal for sucrose formation, could suggest that either both reactions are catalyzed by different enzymes or by different active sites of the same enzyme. Different optimum pH values for both reactions were already reported for the enzyme from sugar beet roots (25). Sucrose formation,

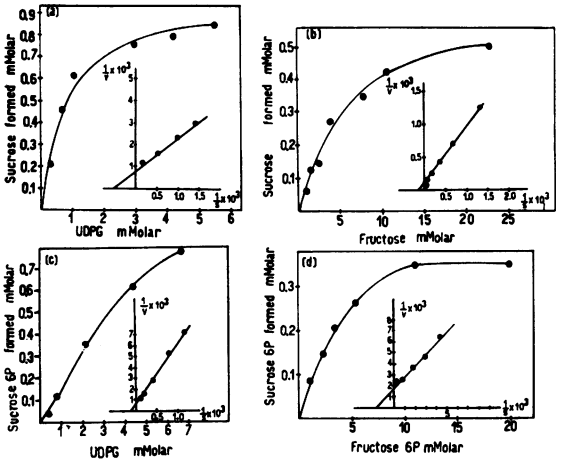

FIG. 4. Effect of substrate concentration on the rate of sucrose and sucrose 6-phosphate synthetase activities: Insets are Lineweaver-Burk double reciprocal plots. The reaction mixture contained, in a final volume of 50 μl, 0.5 μmole of tris maleate buffer, pH 7.5, 20 μl of enzyme (a) and (b); sucrose synthetase; (c) and (d) : sucrose 6-P synthetase. The concentration of UDP-glucose in (b) and (d) was kept constant (0.2 μmole) and in (a) and (c), fructose and fructose 6-P were kept constant. (1 μmole).

when measured with citrate buffer was about 30 % less than with tris buffer.

Effect of Phenolic Glycosides. Of a number of phenolic glycosides tested, β-phenylglycoside, arbutin and salicin at a concentration of 6 mM, were found to be strong competitive inhibitors of UDP sucrose synthetase transferase with Ki values of 9.4×10^{-5}; 1.6×10^{-4} and 2.5×10^{-3} M respectively (fig 5). Phloridzin, *p*-methoxyphenyl gentiobioside and *p*-hydroxyphenyl cellobioside, at the same concentration, did not affect the enzymatic activity.

The activity of sucrose 6-P synthetase, on the contrary was not affected by any of the above mentioned glycosides.

Since the activity of sucrose synthetase can be completely supressed by addition of βphenylglycoside, and the activity of sucrose 6-P synthetase is not affected by this compound, this could be a good way of testing the latter in the presence of the former.

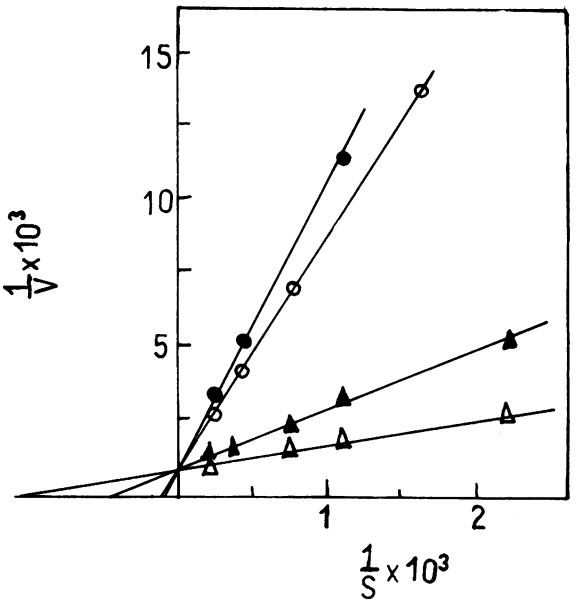

FIG. 5. Kinetics of sucrose formation in the presence and absence of phenyl glycosides: The incubation conditions were as described. The final concentrations of the inhibitors were: β-phenyl glycoside and salicin: 1 mM, and arbutin: 5.5 mM. The concentration of UDP-glucose was varied as indicated. △ None, ○ β-Phenylglycoside, ▲ Salicin, ● Arbutin.

Table III. *^{14}C Incorporation from Sucrose Into Nucleoside Diphosphate Sugars*

The incubation mixture contained, in a final volume of 35 μl, sucrose (100,000 cpm: 8.4 μmoles), tris HCl buffer, 0.5 μmole (pH 7.5 and 6.3) or citrate buffer (pH 6.2); 10 μl of enzyme fraction II and 0.1 μmole of the indicated nucleotide. The incubation was carried out for 60 minutes at 37°. The radioactive sucrose was separated on Whatman No. 1 paper chromatography in butyl alcohol-pyridine-water (6:4:3) and the sugar nucleotide, which remained in the origin in this solvent, was eluted and rechromatographed in ammonium acetate-ethanol (pH 7.5) (27) eluted and counted with a gas flow counter.

Buffer	Addition	Radioactivity incorporated in UDP-glucose	ADP-glucose
		cpm	*cpm*
Tris HCl	None	0	0
	ADP	0	300
	UDP	100	0
Citrate	None	0	0
	UDP	7200	0
	UDP ADP	4500	3000

Effect of Nucleotides. UDP, ADP (at a conc of 3 mM) and ATP (50 mM) produced 60, 50, and 40 % inhibition, respectively of the activity of sucrose synthetase. The synthesis of sucrose 6-P was found to be inhibited by UDP (at the same conc, about 30 %).

Effect of Anions and Cations. The effect of a number of anions and cations on the activity of sucrose and sucrose 6-P is summarized in (fig 6). The decrease in sucrose synthetase activity at higher concentrations of $MgSO_4$ can be explained by the inhibiting effect of the anion.

Effect of Other Substances. Glucose did not modify the activity of sucrose 6-P synthetase but inhibited fructose glucosyl transferase by about 30 % at a concentration of 6 mM. Acids such as chlorogenic and shikimic as well as solanine, at concentrations of 11 mM, 20 mM and 7.6 mM respectively, inhibited the activity of both enzymes to the same

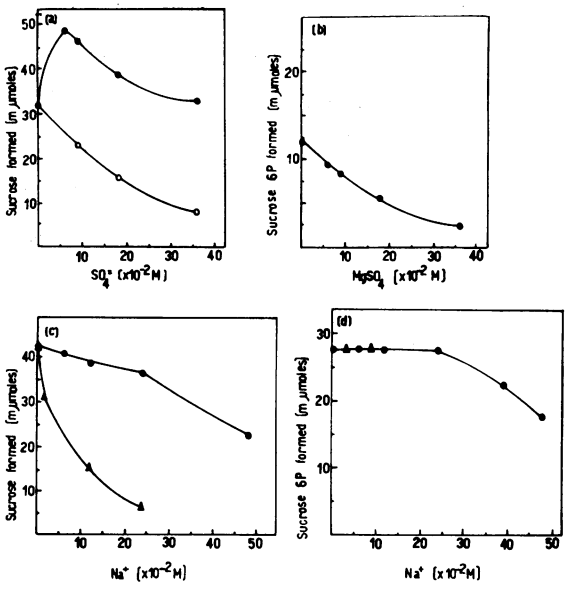

FIG. 6. Effect of anions and cations on sucrose and sucrose 6-P synthetases. The incubation mixture contained, in a final volume of 55 μl, 0 5 μmole tris maleate buffer (pH 7.5); 0.1 μmole of UDP-glucose; 0.5 μmole of fructose or fructose 6-P and 10 μl [(a) and (c)] of sucrose synthetase or [(b) and (d)] of sucrose 6-P synthetase, and the anion or cation at the indicated final concentration. The temperature and incubation time are indicated in Methods. Standard incubations were run without any addition. (a) and (b) : ● $MgSO_4$, ○ Na_2SO_4; (c) and (d) : ● NaCl, ▲ NaF.

extent: between 40 to 60 % the first, 70 to 90 % the second and 20 to 40 % the last. EDTA at a concentration of 10 mM, had no effect on sucrose 6-P synthetase, inhibiting sucrose synthetase by 40 %; *p*-chloromercuri benzoate (0.7 mM) inhibited sucrose formation by almost 70 % and only 25 % the formation of sucrose 6-P.

Discussion

The results described in this paper show clearly that the enzymes, sucrose synthetase, and sucrose 6-P synthetase from potatoes, have quite different properties. Contrary to what has been reported for sucrose and sucrose 6-P synthetase from other sources, potato enzymes had a broad pH optimum, specially sucrose synthetase. Fructose glucosyl transferase was quite resistant to temperature, showing maximum activity when incubated at 45°. A number of phenolic glycosides were found to inhibit sucrose synthetase but had no effect on UDP-glucose fructose 6-P glucosyl transferase.

As was already shown for sucrose synthetase from other sources, Mg^{2+} was found to increase activity. It is difficult to establish if this is absolutely necessary for activity since a high concentration of EDTA (10 mM) inhibits enzyme activity only about 40 %. A more drastic treatment of the enzyme could probably liberate all the bound metal and thus establish if the metal is a cofactor or not.

Sucrose synthetase from potatoes seems to be a sulfydryl enzyme since it is inhibited by *p*-chloromercuribenzoate. However the potato enzyme is less sensitive to this reagent than the synthetase from sugarcane stem (26).

An important difference between the properties of both synthetases described in this work is their different specificity toward the glucosyl donor. Whereas sucrose 6-P synthetase acts only with UDP-glucose, sucrose synthetase can use other sugar nucleotides, specially ADP-glucose which is an effective glucosyl donor. This, and also the fact that ADP inhibits the formation of sucrose competitively while having no effect on sucrose 6-P formation, imply that the role of sucrose synthetase could be a bridge between sucrose and starch formation, whereas the role of sucrose 6-P synthetase would be the synthesis of this disaccharide. The fact that a number of phenolic glycosides which inhibit starch synthesis also inhibit sucrose formation from UDP-glucose or ADP-glucose would speak in favor of a relation between the formation of both sucrose and starch, sucrose 6-P synthetase being excluded from this interconversion (12, 13) and acting solely as sucrose synthesizing enzyme. The way in which sucrose is converted into starch in potato tubers, is still unknown. The formation of sugar nucleotide from sucrose, although occurring in this plant material (table III) under different conditions from those in which sucrose synthetase has its optimum activity, thus bringing into question the identity of the enzyme responsible for the formation of sucrose and the formation of sugar nucleotides from sucrose. This point deserves further investigation.

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