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Sucrose Synthase of Soybean Nodules

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ABSTRACT

Sucrose synthase (UDPglucose: D-fructose 2-α-D-glucosyl transferase, EC 2.4.1.13) has been purified from the plant cytosolic fraction of soybean (*Glycine max* L. Merr cv Williams) nodules. The native enzyme had a molecular weight of 400,000. The subunit molecular weight was 90,000 and a tetrameric structure is proposed for soybean nodule sucrose synthase. Optimum activity in the sucrose cleavage and synthesis directions was at pH 6 and pH 9.5 respectively, and the enzyme displayed typical Michaelis-Menten kinetics. Soybean nodule sucrose synthase had a high affinity for UDP (K_m, 5 micromolar) and a relatively low affinity for ADP (apparent K_m, 0.13 millimolar) and CDP (apparent K_m, 1.1 millimolar). The K_m for sucrose was 31 millimolar. In the synthesis direction, UDPglucose (K_m, 0.012 millimolar) was a more effective glucosyl donor than ADPglucose (K_m, 1.6 millimolar) and the K_m for fructose was 3.7 millimolar. Divalent cations stimulated activity in both the cleavage and synthesis directions and the enzyme was very sensitive to inhibition by heavy metals.

The symbiotic fixation of dinitrogen in root nodules of legumes depends on the supply of photosynthate from the host plant (21). Sucrose is the main carbohydrate translocated into the nodules (4), providing nutrients for the bacteroids, energy for nitrogenase, and carbon skeletons for the assimilation of fixed NH_4^+. The breakdown of sucrose in nodules is thought to take place in the plant cytosolic fraction (21), but there is only limited information on the enzymes involved.

In plant tissues, sucrose may be cleaved by the action of invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) or sucrose synthase (UDPglucose: D-fructose 2-α-D-glucosyltransferase, EC 2.4.1.13) (1, 3, 20). Invertase has been found in soluble extracts of nodules of *Ornithopus sativus*, *Lupinus luteus* (12), *L. angustifolius* (23), and soybeans (26). The properties of soybean nodule alkaline invertase have been described (13). The presence of sucrose synthase in legume nodules was first reported in a recent communication from this laboratory (13).

In the present investigation, a highly purified preparation of sucrose synthase has been obtained from the plant cytosolic fraction of soybean nodules. The enzyme appeared to be one of the major proteins in the nodule cytosol. Soybean nodule sucrose synthase had a high affinity for UDP (K_m, 5 μM) and the K_m for sucrose was 31 mM. In the synthesis reaction, UDPglucose was a more effective glucosyl donor than ADPglucose.

MATERIALS AND METHODS

Materials. Biochemicals were obtained from Sigma Chemical Co. or Boehringer Mannheim GmbH and PBA[2]-60 from Amicon Corp. Perlite was obtained from Australian Perlite Pty. Ltd., Sydney. Nodulaid inoculum (*Rhizobium japonicum* strain CB1809) was a generous gift from Agricultural Laboratories, Sefton, N.S.W. Nodulated soybeans (*Glycine max* L. Merr cv Williams) were grown as described previously (13).

Preparation of Sucrose Synthase. All steps were carried out at 4°C. Nodules (10 g) were harvested 40 d after planting and homogenized with a mortar and pestle in 20 ml of 10 mM K-phosphate (pH 7.2) containing 1 mM EDTA and 5 mM 2-mercaptoethanol (buffer A). A suspension of 10 g of insoluble PVP in 20 ml of buffer A was added to the homogenate, the mixture filtered, and the filtrate centrifuged at 30,000*g* for 15 min. The supernatant was fractionated by the addition of saturated $(NH_4)_2SO_4$ solution, and the fraction which precipitated between 30 and 50% saturation collected by centrifugation at 30,000*g* for 10 min. The precipitate was dissolved in 2.5 ml of buffer A and applied to a Sephadex G-200 column (2.5 × 85 cm) which had been previously equilibrated with 25 mM K-phosphate (pH 7.2) containing 50 mM KCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol. Fractions of 5 ml were collected and those which contained sucrose synthase activity were pooled, dialyzed against buffer A and applied to a DEAE-cellulose column (1.5 × 10 cm) which had been previously equilibrated with buffer A. The column was washed with buffer A until the *A* at 280 nm was less than 0.01 and eluted with a gradient produced by introducing 100 ml of 0.5 M KCl in buffer A into 100 ml of buffer A. Fractions of 4 ml were collected. Active fractions were pooled and dialyzed against 50 mM Hepes-KOH buffer (pH 8.5) containing 5 mM sucrose, 10 mM $MgCl_2$, and 5 mM 2-mercaptoethanol (buffer B). The preparation was applied to a PBA-60 affinity column (1.5 × 5 cm) which had been washed with 250 ml of buffer B containing 200 mM sucrose, followed by 25 ml of buffer B. After unbound protein had been removed, the enzyme was eluted with 0.1 M Tris-HCl buffer (pH 8.5) containing 5 mM 2-mercaptoethanol. Fractions of 3 ml were collected and those which contained activity were pooled and dialyzed against 20 mM K-phosphate (pH 7.0) containing 5 mM 2-mercaptoethanol. Preparations of this type were used to study the properties of soybean nodule sucrose synthase.

Assay of Sucrose Synthase Activity. All assays were carried out at 30°C. Activity in the sucrose cleavage direction was assayed by three methods (assays A–C). Three other methods were used to assay synthesis activity (assays D–F).

Assay A. The production of UDPglucose was coupled to the reduction of NAD in the presence of excess UDPglucose dehydrogenase and the change in *A* at 340 nm followed. Reaction mixtures contained in a volume of 1 ml, 20 μmol Hepes-KOH

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[2] Abbreviations: PBA, phenyl boronate agarose; Ches, 2-cyclohexylaminoethanesulfonic acid; U, unit; *V*, maximum velocity; K_i, dissociation constant.

buffer (pH 7.5), 100 μmol sucrose, 2 μmol UDP, 1.5 μmol NAD, 25 μg UDPglucose dehydrogenase, and an appropriate volume of enzyme. Assay A was used to study the kinetic parameters and the effects of pH, fructose, metabolites, and salts on the cleavage reaction.

Assay B. The effect of inhibitors on sucrose cleavage was studied in reaction mixtures which contained, in a volume of 1 ml, 20 μmol Hepes-KOH buffer (pH 7.5), 100 μmol sucrose, 2 μmol UDP, and an appropriate volume of enzyme. The reaction was stopped after 30 min by heating in a boiling water bath for 2 min, and fructose was determined by the reducing sugar method of Nelson (16).

Assay C. Sucrose cleavage activity with glucosyl acceptors other than UDP, and the inhibition of sucrose cleavage by UDPglucose, were studied in reaction mixtures which contained, in a volume of 1 ml, 20 μmol Hepes-KOH buffer (pH 7.5), 100 μmol sucrose, 2 μmol nucleotide diphosphate, and an appropriate volume of enzyme. The reaction was stopped after 30 min by heating in boiling water bath for 2 min. Fructose was determined from the change in *A* at 340 nm following the addition of a solution which contained, in a volume of 0.2 ml, 70 μmol Tris-HCl buffer (pH 7.5), 60 μmol KCl, 2 μmol $MgCl_2$, 0.36 μmol NADP, 1.2 μmol ATP, 20 μg hexokinase, 20 μg P-glucose isomerase, and 10 μg glucose-6-P dehydrogenase.

Assay D. Reaction mixtures contained, in a volume of 1 ml, 20 μmol Hepes-KOH buffer (pH 7.5), 15 μmol fructose, 2 μmol UDPglucose, and an appropriate volume of enzyme. The reaction was stopped after 30 min by heating for 2 min in a boiling water bath. UDP was determined from the change in *A* at 340 nm following the addition of a solution which contained, in a volume of 0.2 ml, 5 μmol $MgCl_2$, 0.4 μmol P-enolpyruvate, 0.15 μmol NADH and 20 μmol KCl, 25 μg pyruvate kinase, and 25 μg lactate dehydrogenase. This assay was used to study the effects of pH, sucrose, metabolites, salts, and inhibitors on the synthesis reaction. When the effect of pH was studied, 0.12 mmol Hepes-KOH buffer (pH 7.5) was included in the solution added after the reaction was stopped.

Assay E. Activity was assayed by coupling the production of nucleotide diphosphate to the oxidation of NADH in the presence of excess pyruvate kinase and lactate dehydrogenase. Reaction mixtures contained, in a final volume of 1 ml, 20 μmol Hepes-KOH buffer (pH 7.5), 15 μmol fructose, 2 μmol UDP-glucose, 5 μmol $MgCl_2$, 0.4 μmol P-enolpyruvate, 0.15 μmol NADH, 20 μmol KCl, 25 μg pyruvate kinase, 25 μg lactate dehydrogenase, and an appropriate volume of enzyme. The decrease in *A* at 340 nm was followed. The effect of ADPglucose was studied in reaction mixtures of the same composition except that UDPglucose was omitted and the concentration of ADPglucose varied.

Assay F. Reaction mixtures were of the composition described for assay D. The reaction was stopped after 30 min by the addition of 0.1 ml of 0.1 M citrate buffer (pH 5.0) and heating in a boiling water bath for 2 min. Glucose was determined according to the method of Blakeney and Matheson (5) following the addition of 0.1 mg of invertase. Assay F was used to study the inhibition of sucrose synthesis by UDP and ADP.

One U of activity is defined as the amount of enzyme which catalyzed the formation of 1 μmol of product/min. Protein was determined by the Folin-Lowry method.

Analysis of Data. The kinetic constants for UDP, UDPglucose, sucrose, and fructose were determined by fitting the data to the appropriate rate equation as described by Cleland (6). The equations used were:

$$v = \frac{VA}{K_{ia}K_b + K_bA + K_aB + AB} \quad (1)$$

$$v = \frac{VA}{K_a + A + A/K_i} \quad (2)$$

Kinetic constants for ADP, CDP, and ADPglucose were calculated by nonlinear regression analysis of initial velocity data as described by Duggleby (9).

Gel Electrophoresis. PAGE was carried out according to the method of Davis (7) and gels were stained for protein (10) and carbohydrate (19) as described. Sucrose synthase activity was located by incubating gels at 37°C for 30 min in a reaction mixture which contained, in a volume of 10 ml, 0.2 mmol Hepes-KOH buffer (pH 7.5), 1 mmol sucrose, and 20 μmol UDP. The gels were rinsed in distilled H_2O and incubated in 1 N NaOH containing 1% (w/v) triphenyl tetrazolium chloride for 10 min at 37°C. The mol wt of sucrose synthase was estimated by PAGE according to the method of Hedrick and Smith (11), using thyroglobulin, ferritin, catalase, lactate dehydrogenase, and BSA as standards. SDS-PAGE gel electrophoresis was carried out as described by Weber and Osborn (30). Phosphorylase b, BSA, ovalbumin, carbonic anhydrase, trypsin inhibitor, and α-lactalbumin were used as standards.

Mol Wt Determination by Gel Filtration. The mol wt of sucrose synthase was determined in a Sephadex G-200 column according to the method of Andrews (2). Ferritin, catalase, aldolase, BSA, and Cyt *c* were used as calibration proteins.

RESULTS

Sucrose synthase was purified 23-fold from the plant cytosolic fraction of soybean nodules using the procedure summarized in Table I. The enzyme could be stored without loss of activity for 4 weeks at 4°C in 20 mM K-phosphate (pH 7) containing 5 mM 2-mercaptoethanol. There was no cleavage of sucrose in the absence of UDP and the preparation was free of sucrose-P synthase activity.

A single band was observed in gels that were stained for sucrose synthase activity following electrophoresis of the crude plant soluble fraction of soybean nodules (Fig. 1). The active band had the same mobility as one of the prominent protein bands in the gels (Fig. 1). PAGE of the purified soybean nodule sucrose synthase showed the presence of one major protein band as well as a faint band of higher mobility (Fig. 1). The major protein band coincided with single bands detected when gels of the purified enzyme were stained for sucrose synthase activity and carbohydrate (Fig. 1).

The mol wt of the native enzyme, as determined by PAGE, was 380,000 ± 20,000. A value of 412,000 ± 25,000 was obtained by gel filtration. A single polypeptide band with a mol wt of 90,000 ± 2,000 was observed when the soybean nodule sucrose synthase was subjected to SDS-PAGE.

Effect of pH. The effect of pH on soybean nodule sucrose synthase is shown in Figure 2. Optimum activity in the cleavage direction was at pH 6 and activities of 90% or greater of the maximum were observed between pH 5 and pH 7. In the synthesis direction optimum activity was at pH 9.5 and activities of 90% or greater than the maximum were observed between pH 8.5 and 10 (Fig. 2). The properties of sucrose synthase were investigated at pH 7.5 to enable a direct comparison of the synthesis and cleavage activities of the enzyme at a pH close to values that have been reported for the cytoplasm in roots (22). At pH 7.5, the cleavage and synthesis activities of soybean nodule sucrose synthase were similar.

Kinetic Constants of Soybean Nodule Sucrose Synthase. Typical Michaelis-Menten kinetics were observed for soybean nodule sucrose synthase in the cleavage and synthesis reactions. When the concentration of sucrose was varied at different concentrations of UDP an intersecting pattern of linear double reciprocal plots was obtained (Fig. 3). Replots of the slopes and intercepts

Table I. *Purification of Soybean Nodule Sucrose Synthase*

Soybean nodules (9 g) were extracted and sucrose synthase activity measured as described using assay A.

Stage	Volume	Protein Content	Activity	Specific Activity	Recovery
	ml	*mg*	*U*	*U/mg*	*%*
Crude extract	51	69.1	46.5	0.67	100
30–50% $(NH_4)_2SO_4$	3.9	34.6	44.6	1.29	96
Sephadex G-200	41.5	6.4	35.7	5.6	77
DEAE-cellulose	34.0	2.4	28.6	12.0	62
PBA-60	7.8	1.5	22.2	15.1	48

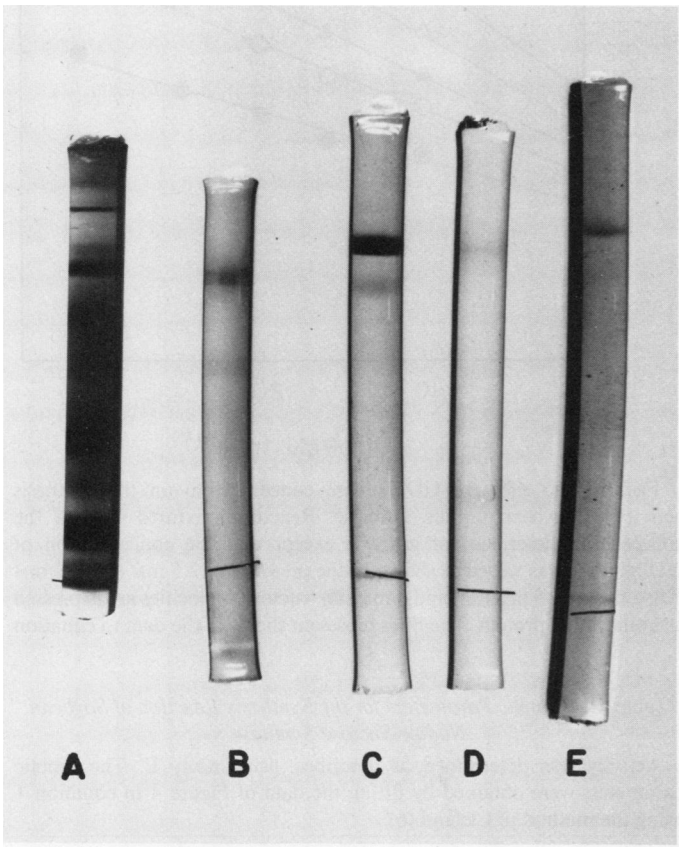

FIG. 1. Electrophoresis of soybean nodule sucrose synthase. Electrophoresis was carried out in 6% (w/v) polyacrylamide gels as described, with 100 μg of protein from the nodule cytosolic extract applied in gels A and B and 5 μg of the purified enzyme in gels C, D, and E. Gels A and C were stained for protein, gels B and D for sucrose synthase activity, and gel E for carbohydrate as described.

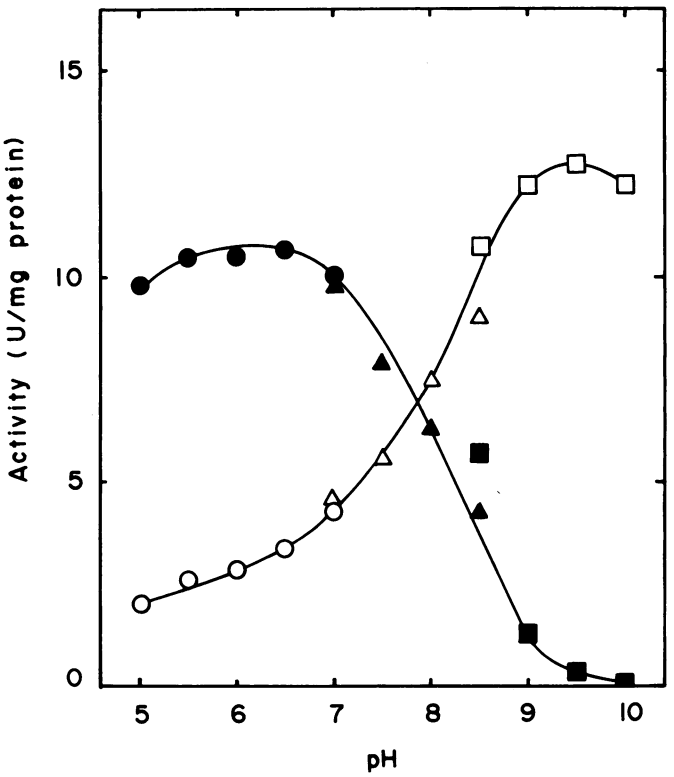

FIG. 2. Effect of pH on soybean nodule sucrose synthase. Activities in the cleavage and synthesis directions were determined as described using assays A and D, respectively, except that 20 μmol Mes-KOH (●, ○), 20 μmol Hepes-KOH (▲, △) and 20 μmol Ches-KOH (■, □) buffers were used. (●, ▲, ■), Cleavage activity; (○, △, □), synthesis activity.

were linear and a good fit of the data was obtained with the equation describing an intersecting initial velocity pattern (Eq. 1). The kinetic constants for the cleavage reaction are shown in Table II.

An intersecting pattern of linear double reciprocal plots was observed when the concentration of UDPglucose was varied at the concentrations of fructose shown in Figure 4. The slope and intercept replots were linear. Kinetic constants for the synthesis reaction are given in Table III. At concentrations of fructose greater than 15 mM, substrate inhibition of the synthesis reaction occurred. The substrate inhibition by fructose was studied by varying the concentration of fructose in the presence of 2 mM UDPglucose. The data (not shown) fitted well to the equation for linear substrate inhibition (Eq. 2), and a value of 26 ± 2 mM was obtained for the inhibition constant for fructose.

Substrate Specificity. Michaelis-Menten kinetics were observed when ADP and CDP acted as glucosyl acceptors in the cleavage reaction. Table IV shows that the apparent K_m values for ADP and CDP were 0.13 ± 0.01 and 1.1 ± 0.1 mM, respectively. The values of the apparent V for ADP and CDP as glucosyl acceptors were, respectively, 10- and 20-fold lower than the V with UDP. The constants given for ADP and CDP are apparent values as the concentration of sucrose in the assays was not saturating. The sucrose cleavage activity with GDP (final concentration, 2 mM) as glucosyl acceptor was 0.5% of the rate with UDP.

In the synthesis reaction, the enzyme followed Michaelis-Menten kinetics when ADPglucose acted as the glucosyl donor. Values of 1.6 ± 0.1 mM and 1.95 ± 0.05 U/mg protein were determined for the apparent K_m and apparent V, respectively. These are only apparent values as saturating, noninhibitory concentrations of fructose could not be achieved in the assays.

Inhibition by Products. Cleavage activity of soybean nodule sucrose synthase was inhibited 50% by 9 mM fructose and 15 mM UDPglucose, respectively. Synthesis activity was strongly inhibited by UDP with inhibitions of 25% and 50% being given by 0.3 and 0.7 mM UDP, respectively. ADP (final concentration, 10 mM) inhibited sucrose synthesis 50% and sucrose (100 mM)

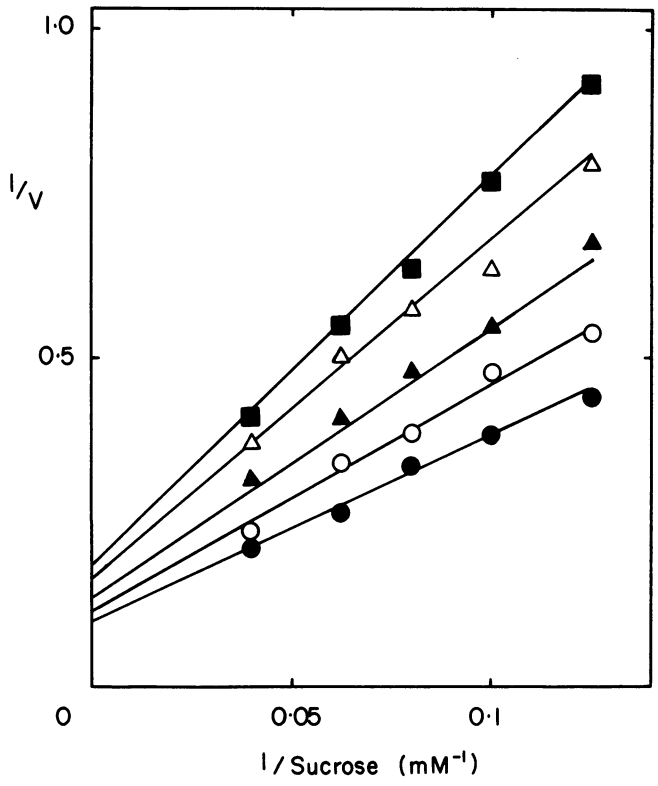

FIG. 3. The effect of sucrose concentration on the cleavage activity of soybean nodule sucrose synthase. Reaction mixtures were of the composition described for assay A except that the concentration of sucrose was varied as shown in the presence of 3.2 μM (●), 4 μM (○), 6.25 μM (▲), 10 μM (△), and 20 μM (■) UDP. Velocities are expressed as units/mg of protein. The lines represent the fit of the data to equation 1.

Table II. *Kinetic Parameters for the Cleavage Reaction of Soybean Nodule Sucrose Synthase*

Activity was determined as described using assay A. The kinetic parameters were obtained by fitting the data of Figure 3 to equation 1 using the method of Cleland (6).

Parameter	Value
V (U/mg protein)	13.3 ± 2.0
K_m sucrose (mM)	31.3 ± 7.1
K_i sucrose (mM)	31.9 ± 13.1
K_m UDP (mM)	0.005 ± 0.002
K_i UDP (mM)	0.005 ± 0.001

inhibited the synthesis reaction by 25%.

Effect of Metabolites. Soybean nodule sucrose synthase was inhibited by glucose. In the cleavage direction, activity was inhibited 25% and 50% by glucose concentrations of 2 and 5 mM, respectively. The synthesis reaction was inhibited 25% by 5 mM glucose. There was no effect on the cleavage activity by UTP (final concentration, 5 mM), ATP (5 mM), or allantoin (5 mM). UTP (5 mM) and ATP (5 mM) inhibited the sucrose synthesis reaction by 50% and 15%, respectively, while allantoin (5 mM) stimulated synthesis activity by 43%. The following metabolites (final concentration, 5 mM) had no effect on the cleavage or synthesis reactions: galactose, mannose, maltose, raffinose, glucose-1-P, glucose-6-P, fructose-6-P, fructose-1,6-P_2, 3-P-glycerate, P-enolpyruvate, ethanol, succinate, 2-oxoglutarate, glutamate, glutamine, NAD, AMP, and PPi.

Effect of Salts. Na^+, K^+, and NH_4^+ (final concentration, 50 mM), added as the Cl^- salts, had no effect on the cleavage or synthesis reactions of soybean nodule sucrose synthase. The

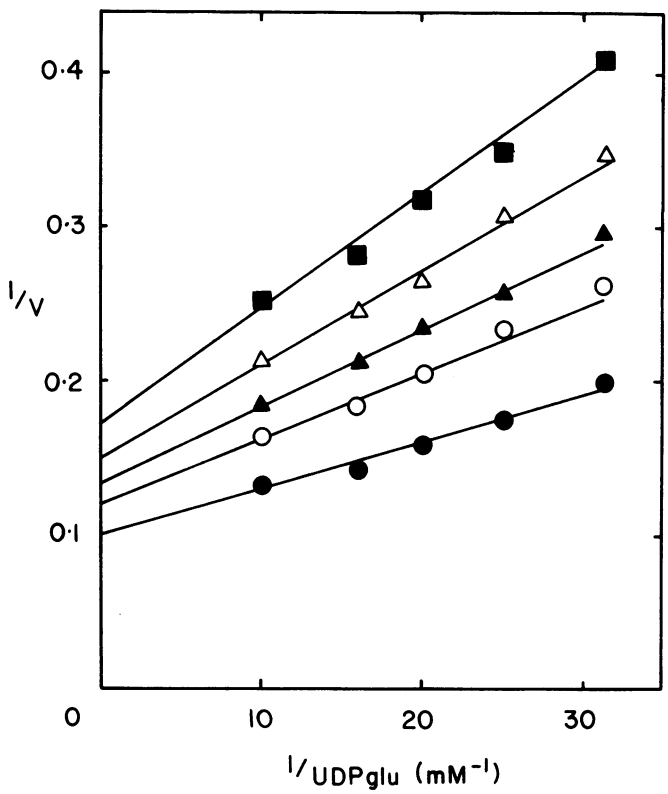

FIG. 4. The effect of UDPglucose concentration on the synthesis activity of soybean nodule synthase. Reaction mixtures were of the composition described for assay E except that the concentration of UDPglucose was varied as shown in the presence of 2.5 mM (●), 3.2 mM (○), 4 mM (▲), 5 mM (△), and 8 mM (■) fructose. Velocities are expressed as units/mg of protein. The lines represent the fit of the data to equation 1.

Table III. *Kinetic Parameters for the Synthesis Reaction of Soybean Nodule Sucrose Synthase*

Activity was determined as described using assay E. The kinetic parameters were obtained by fitting the data of Figure 4 to equation 1 using the method of Cleland (6).

Parameter	Value
V (U/mg protein)	14.3 ± 1.2
K_m fructose (mM)	3.7 ± 0.8
K_i fructose (mM)	19.6 ± 9.9
K_m UDPglucose (mM)	0.012 ± 0.006
K_i UDPglucose (mM)	0.064 ± 0.014

Table IV. *Nucleotide Specificity of Soybean Nodule Sucrose Synthase*

Activity was determined as described using assay C. The kinetic constants were calculated by weighted nonlinear regression of the initial rate data using the method of Duggleby (9).

Nucleotide	Apparent K_m	Apparent V
	mM	*U/mg protein*
ADP	0.13 ± 0.01	1.38 ± 0.03
CDP	1.1 ± 0.1	0.71 ± 0.03

following anions (50 mM), added as the Na^+ or K^+ salts, had no effect on the enzyme in either reaction direction: Cl^-, Br^-, F^-, NO_3^-, Pi, SO_4^{2-}, borate, acetate, and citrate.

Mg^{2+} and Ca^{2+} had a small stimulatory effect on sucrose

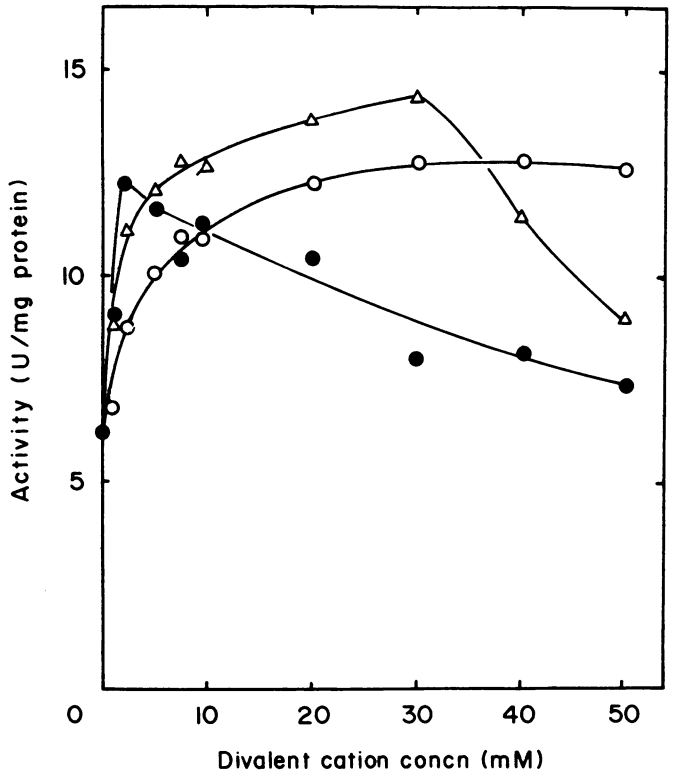

FIG. 5. Effect of divalent cations on the synthesis activity of soybean nodule sucrose synthase. Activity was determined using assay D as described, with $MgCl_2$ (○), $CaCl_2$ (△), and $MnCl_2$ (●) included in the reaction mixtures at the concentrations shown.

cleavage activity. The maximum stimulation of 30% occurred when 10 mM $MgCl_2$ or $CaCl_2$ were included in reaction mixtures. Mn^{2+} inhibited the cleavage reaction, with inhibitions of 25% and 50% being given by $MnCl_2$ concentrations of 10 and 20 mM, respectively. The effect of Mg^{2+}, Ca^{2+}, and Mn^{2+} on sucrose synthesis activity is shown in Figure 5. All stimulated activity by a maximum of approximately 2-fold, but the degree of stimulation given by Mn^{2+} and Ca^{2+} decreased at concentrations above 2 and 30 mM, respectively (Fig. 5).

Effect of Inhibitors. Soybean nodule sucrose synthase was inhibited by heavy metals. In the cleavage direction, activity was inhibited 50% by 1 μM $HgCl_2$ or $CuSO_4$. The cleavage reaction was inhibited 50% by 0.9 μM $HgCl_2$ and 6 μM $CuSO_4$, respectively. DTT (final concentration, 5 mM), GSH (5 mM), 2-mercaptoethanol (5 mM), and EDTA (50 mM) had no effect on the cleavage or synthesis reactions. Both sucrose cleavage and synthesis were inhibited 60% by 20 mM imidazole-HCl. Tris-HCl (20 mM) inhibited the cleavage reaction by 25% but had no effect on the synthesis reaction.

Equilibrium Constant. A value of 0.39 was determined at pH 7.5 for the equilibrium constant, K = [fructose][UDPglucose]/[sucrose][UDP].

DISCUSSION

Soybean nodules contained a single form of sucrose synthase. The enzyme appeared to be one of the major proteins in the plant cytosolic fraction as shown by electrophoresis of crude soybean nodule extracts. Furthermore, a purification of only 23-fold yielded a preparation of the sucrose synthase which was close to homogeneity. Sucrose synthase is one of the major soluble proteins in maize kernels (27), and the enzyme constitutes 1% of the soluble protein in bamboo shoots (28).

The mol wt of soybean nodule sucrose synthase was near 400,000. The purified enzyme appeared to be associated with carbohydrate and this may account for the difference in the values of the mol wt obtained by PAGE and gel filtration. The subunit mol wt of soybean nodule sucrose synthase was 90,000, indicating that the native enzyme is probably a tetramer. The sucrose synthases of maize kernels (27), mung bean seedlings (8), and rice grains (17) have tetrameric structures with mol wt near 400,000.

Sucrose synthase of soybean nodules followed typical Michaelis-Menten kinetics, and the addition of substrates to the enzyme in the cleavage and synthesis reactions was sequential. Michaelis-Menten kinetics have been displayed by a number of other sucrose synthases, although sigmoidal kinetics have been reported for the enzyme from sweet potato (14), potato tubers, rice grains (15), and bamboo shoots (28). Divalent cations were required for maximum activity in both the cleavage and synthesis reactions. The stimulation of sucrose synthesis by divalent cations has been reported for other sucrose synthases (8, 18, 29). Heavy metals were potent inhibitors of the cleavage and synthesis reactions, suggesting the involvement of a sulfhydryl group in catalysis.

Soybean nodule sucrose synthase had a high affinity for UDP. The K_m for UDP (5 μM) was considerably lower than the range of values (0.06–8.3 mM) that have been reported for other sucrose synthases (1, 3, 20). ADP could act as the glucosyl acceptor in the cleavage reaction but was much less effective than UDP. The apparent K_m for ADP was 26-fold higher than the K_m for UDP and the apparent V with ADP was 10-fold less than the V with UDP. CDP was even less effective as the glucosyl acceptor and the enzyme had negligible activity with GDP. Sucrose synthase is thus unlikely to be significant in the formation of ADPglucose and CDPglucose in soybean nodules. UDP is generally the preferred glucosyl acceptor for sucrose synthases (1, 3, 20). In the synthesis reaction, UDPglucose was more effective as the glucosyl donor than ADPglucose. The affinity of soybean nodule enzyme for UDPglucose was high compared to other sucrose synthases (1, 3, 20).

Soybean nodules contain high concentrations of sucrose and very low concentrations of fructose (24, 25). Thus, although the reaction catalyzed by sucrose synthase is readily reversible, the enzyme is likely to act predominantly in the breakdown of sucrose in soybean nodules. The higher affinity of the enzyme for UDP than for UDPglucose, and the strong inhibition of sucrose synthesis by UDP, would also favor the cleavage reaction. Inhibition of the cleavage reaction by the products UDPglucose and fructose is unlikely to be metabolically significant, but there may be inhibition by glucose *in vivo*. Streeter (25) has shown that the mean concentration of glucose in soybean nodules is 1.4 mg/g fresh weight.

The plant cytosolic fraction of soybean nodules contains alkaline invertase and sucrose synthase (13). Both enzymes appear to be regulated mainly by the availability of sucrose. Alkaline invertase (K_m, 10 mM) (13) has a higher affinity for sucrose than sucrose synthase (K_m, 31 mM) and this may determine how sucrose in the nodules is partitioned between the two reactions. The simultaneous action of alkaline invertase and sucrose synthase in the nodule cytosol would ensure that, while most of the sucrose is converted to hexoses for further catabolism, some UDPglucose would be directly available for the formation of other nucleotide sugars and polysaccharides.

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