Purification and Biochemical Characterization of Sucrose Synthase from the Cytosolic Fraction of Chickpea (*Cicer arietinum* L. cv. Amethyst) Nodules

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Sucrose synthase (EC 2.4.1.13) has been purified from the plant cytosolic fraction of chickpea (*Cicer arietinum* L. cv. Amethyst) nodules. The native enzyme had a molecular mass of 356±15 kD. The subunit molecular mass was 87±2 kD, and a tetrameric structure is proposed for sucrose synthase of chickpea nodule. Optimum activities in the sucrose cleavage and synthesis directions were at pH 6.5 and 9.0, respectively. The purified enzyme displayed typical hyperbolic kinetics with substrates in cleavage and synthesis reactions. Chickpea nodules sucrose synthase had a high affinity for UDP (K_m, 8.0 μM) and relatively low affinities for ADP (K_m, 0.23 mM), CDP (K_m, 0.87 mM), and GDP (K_m, 1.51 mM). The K_m for sucrose was 29.4 mM. In the synthesis reaction, UDP-glucose (K_m, 24.1 μM) was a more effective glucosyl donor than ADP-glucose (K_m, 2.7 mM), and the K_m for fructose was 5.4 mM. Divalent cations, such as Ca^{2+}, Mg^{2+}, and Mn^{2+}, stimulated the enzyme activity in both the cleavage and synthesis directions, and the enzyme was very sensitive to inhibition by $HgCl_2$ and $CuSO_4$.

Key words : *chickpea nodule,* Cicer arietinum, *sucrose synthase, cleavage, synthesis.*

The symbiotic fixation of dinitrogen in leguminous root nodules depends on the supply of photosynthate from the host plant.[1] Sucrose is the major carbohydrate translocated into the nodules,[2] providing nutrients for the bacteroids, energy for nitrogenase, and carbon skeletons for the assimilation of fixed ammonia. The breakdown of sucrose in nodules is thought to be taken place in the plant cytosolic fraction,[1] but there is only limited information on the enzymes involved. Furthermore, much less is known of carbon metabolism in indeterminate nodules, which differ from determinate nodules morphologically and in the nature of nitrogenous compounds exported into the xylem.[3]

In plant tissues, sucrose may be cleaved by the action of invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) or sucrose synthase (UDP-glucose: D-fructose 2-α-D-glucosyltransferase, EC 2.4.1.13). Invertase irreversibly hydrolyzes sucrose to glucose and fructose,[4] and has been found in soluble extracts of nodules of *Ornithopus sativus*,[5] *Lupinus angustifolius*,[6] and soybeans.[7] The properties of soybean nodule alkaline invertase have been described by Morell and Copeland.[7] Sucrose synthase catalyzes the transfer of the glucose residue from sucrose to UDP, yielding UDP-glucose and fructose. The enzyme has been characterized in various plant species.[8-10] Sucrose synthase is a tetrameric with a native molecular mass in the range of 280 to 400 kDa. It has optimal activity in the cleavage direction between pH 6.0 and 8.5. In the direction of sucrose synthesis, pH 8.5 to 9.5 was found to be optimal.[11] The K_m values of sucrose synthase differ considerably from plant to plant, and are in the ranges of 10 to 290 mM for sucrose and 0.05 to 6.6 mM for UDP. The presence of sucrose synthase in indeterminate nodules was first reported in a communication from this laboratory.[12] In the present report, a highly purified preparation of sucrose synthase has been obtained from the cytosolic fraction of chickpea nodules, and, therefore, biochemical properties of sucrose synthase have been described.

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Materials and Methods

Materials. Chickpea (*Cicer arietinum* L. cv. Amethyst) seeds were surface sterilized in 0.4% (w/v) sodium hypochlorite for 10 min, rinsed thoroughly with running tap water for 15 min, inoculated with *Rhizobium* sp. (*Cicer*) CC1192, and sown in moistened perlite in pots at a depth of approximately 2 cm and 3-4 cm apart. Plants were grown in glasshouse with average day and night temperatures of 25 and 19°C, respectively. N-free nutrient solution[13] was given to the plants every 3-4 days. Q-Sepharose, Sephadex G-200, and Superose 6 Prep Grade were purchased from Pharmacia, Uppsala, Sweden, and PBA-60 from Amicon Corp. Other biochemicals were of Sigma Chemicals Co. St Louis, MO, USA, or Boehringer-Mannheim GmbH, Mannheim, Germany.

Purification of sucrose synthase. If not stated otherwise, all steps were carried out in 4°C. Nodules (25 g) from 38- to 43-day-old chickpea plants were rinsed with distilled water and homogenized with a mortar and pestle in 3 volumes of an ice-cold extraction buffer A (30 mM K-phosphate, pH 7.2, containing 1 mM EDTA, 100 mM mannitol, and 5 mM 2-mercaptoethanol). A suspension of 10 g of insoluble PVP in 75 ml of buffer A was added to the homogenate, the homogenate was squeezed through a single layer of Miracloth (Calbiochem, San Diego, CA, USA), and the filtrate was centrifuged at 25,000 g for 15 min. Solid ammonium sulfate was slowly added to the crude extract with gentle stirring, and the protein that precipitated between 30 and 50% was collected by centrifugation at 20,000 g for 10 min. The precipitate was dissolved in 3 ml of buffer B (30 mM K-phosphate, pH 7.2, containing 50 mM KCl, 1 mM EDTA, and 5 mM 2-mercaptoethanol). The protein solution was applied to a Sephadex G-200 column (100×2.5 cm) which had been equilibrated previously with buffer B and calibrated with blue dextran 2000 (V_o), thyroglobulin (669 kD), ferritin (450 kD), catalase (240 kD), aldolase (158 kD), and BSA (66 kD). The chromatography was performed at room temperature (20 to 22°C) using a flow rate of 2.5 ml min^{-1}. The fractions which contained sucrose synthase activity were pooled, dialyzed against buffer C (10 mM K-phosphate, pH 7.2, containing 1 mM EDTA and 5 mM 2-mercaptoethanol) and applied to a Q-Sepharose column (25×2.5 cm) which had been equilibrated previously with buffer C. The column was washed with buffer C until the absorbance at 280 nm was less than 0.01 and eluted with a gradient produced by introducing 100 ml of buffer C containing 0.5 M KCl into 100 ml of buffer C. The flow rate was 1.5 ml min^{-1}, and fractions of 4 ml were collected. Fractions containing the enzyme activity were pooled and dialyzed overnight against 2 L of 50 mM HEPES-KOH, pH 8, containing 5 mM sucrose, 10 mM $MgCl_2$, and 5 mM 2-mercaptoethanol (buffer D). The dialyzed protein solution was applied to PBA-60 affinity column (10×1 cm) which had been equilibrated previously with buffer D containing 200 mM sucrose and washed with 30 ml of buffer D. After unbound protein had been removed, the enzyme was eluted with 0.1 M Tris-HCl buffer (pH 7.5) containing 5 mM 2-mercaptoethanol. Fractions containing the enzyme activity were pooled and dialyzed for 2 hrs against 2 L of buffer B, and concentrated overnight to 0.5 ml in a dialysis bag which was covered with sucrose. For further purification, the concentrated enzyme from the PBA-60 step was chromatographed at 20 to 22 through a Superose 6 Prep Grade column (47×1 cm) using a flow rate of 1.5 ml min^{-1} in buffer B. Active fractions were pooled and dialyzed against 2 L of 20 mM K-phosphate (pH 7.0) containing 5 mM 2-mercaptoethanol for 2 hrs. Preparation of this type was used to study the properties of chickpea nodule sucrose synthase.

Enzyme activity. All enzyme assays were performed at 30 in reaction mixtures. Activity in the sucrose cleavage direction was assayed by three methods (assays A-C). Three other methods were used to assay synthesis activity (assays C-E). *Assay A*: The production of UDP-glucose was coupled to the reduction of NAD^+ in the presence of excess UDP-glucose dehydrogenase and the change in the absorbance at 340 nm followed. Reaction mixture (1 ml) contained 20 mM HEPES-KOH buffer (pH 7.5), 100 mM sucrose, 2 mM UDP, 1.5 mM NAD, 25 μg UDP-glucose dehydrogenase (type III, Sigma), and an appropriate volume of the 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 effects of various inhibitors on sucrose cleavage were studied in a reaction mixture (1 ml) containing 20 mM HEPES-KOH buffer (pH 7.5), 100 mM sucrose, 2 mM UDP, and an appropriate volume of the enzyme. The reaction was carried out at 30°C and was stopped after 30 min by heating in a boiling water bath for 2 min. Fructose was determined by the reducing sugar method of Nelson.[14] *Assay C*: Sucrose cleavage activity with glucosyl acceptors other than UDP and the inhibition of sucrose cleavage by UDP-glucose were studied in a reaction mixture (1 ml) containing 20 mM HEPES-KOH buffer (pH 7.5), 100 mM sucrose, 2 mM nucleotide diphosphate, and an appropriate volume of the enzyme. The reaction was carried out at 30°C and was stopped after 30 min by boiling the sample for 2 min. Fructose was determined by the method described by Morell and Copeland.[9] *Assay D*: Reaction mixture (1 ml) contained 20 mM HEPES-KOH (pH 7.5), 15 mM fructose, 2 mM UDP-glucose, and an appropriate volume of the enzyme. The reaction was carried out at 30°C and was stopped after 30 min by boiling the sample for 2 min. UDP was determined by measuring the decrease in absorbance at 340 nm after the addition of a solution (0.2 ml) containing 5 mM $MgCl_2$, 0.4 mM phosphoenolpyruvate, 0.15 mM NADH, 20 mM KCl, 25 μg pyruvate kinase, and 25 lactate dehydrogenase. This assay was used to study the effects of pH, sucrose, metabolites, salts, and inhibitors on the synthesis reaction. *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 mixture (1 ml) contained 20 mM HEPES-KOH buffer (pH 7.5), 15 mM fructose, 2 mM UDP-glucose, 5 mM $MgCl_2$, 0.4 mM phosphoenolpyruvate, 0.15 mM NADH, 20 mM KCl, 25 μg pyruvate kinase, 25 μg lactate dehydrogenase, and an appropriate volume of the enzyme. The decrease in the absorbance at 340 nm was followed. The effect of ADP-glucose was studied in reaction mixtures of the same composition except that UDP-glucose was omitted and the concentration of ADP-glucose varied. *Assay F*: Reaction mixtures were of the composition described for assay D. The reaction was stopped after 30 min by adding 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[15]. Assay D was used to study the inhibition of sucrose synthesis by UDP and ADP. One unit of the activity is defined as the amount of enzyme which catalyzed the formation of 1 μmol of product/min. Protein was determined by the Pierce Protein Assay, according to the manufacturer's protocol.

Electrophoresis. SDS-PAGE with gels of 12.6% polyacrylamide was carried out by the procedure of King and Laemmli[16] with a minigel apparatus (Bio-Rad). Separated proteins were visualized by staining with Coomassie brilliant blue R-250. In order to detect N-linked glycoproteins, the nitrocellulose membrane was decorated with concanavalin A or an antiserum against Xyl-containing complex glycans, as described by Faye and Chrispeels[17] and Laurière *et al.*[18]

Analysis of data. The kinetic constants for UDP, UDP-glucose, sucrose, and fructose were determined by fitting the data to the appropriate rate equation as described by Cleland.[19] The equations used were:

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

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

Kinetic constants for ADP, CDP, and ADP-glucose were calculated by nonlinear regression analysis of initial velocity data as described by Duggleby.[20]

Results

Sucrose synthase was purified to a specific activity of 16.4 unit/mg protein with an overall recovery of 17% from the cytosolic fraction of chickpea nodules using the procedure shown in Table 1. The enzyme was eluted in a single peak from the Q-Sepharose column with maximum activity corresponding to a KCl concentration of 0.19 M. However, subsequent chromatographic steps were carried on PBA-60 and Superose 6 Prep Grade columns because several polypeptide bands were detected on SDS-PAGE after the step of Q-Sepharose column chromatography. A single polypeptide band of sucrose synthase on SDS-PAGE were observed from a single peak of Superose 6 Prep Grade column chromatography. The purified enzyme was stored without loss of activity for 3 weeks at 4°C in 20 mM K-phosphate buffer (pH 7.0) containing 5 mM 2-mercaptoethanol. There was no cleavage of sucrose in the absence of UDP, and the preparation was free of the activity of sucrose phosphate synthase.

The native molecular mass of sucrose synthase, as determined by gel filtration through Sephadex G-200 and Superose 6 Prep Grade, was approximately 356±15 kD (means±SE of 3 determinations). A single polypeptide band with molecular mass of 87 kD (means±SE of 3 determinations) was stained with Coomassie Blue reagent when the most highly purified preparation of sucrose synthase prepared from Superose 6 Prep Grade column chromatography was subjected to SDS-PAGE (Fig. 1). Therefore, the enzyme appears to be a tetramer, as are other plant sucrose synthases.[8,9,21] To test whether the purified sucrose synthase was N-linked glycoprotein, it was decorated on western blots with concanavalin A or an antibody against Xyl-containing complex N-linked glycans obtained from plant tissues. Neither the lectin nor the anti-carbohydrate antibody bound to the proteins of sucrose synthase (data not shown). This result implies that the sucrose synthase of chickpea nodule is not N-glycosylated.

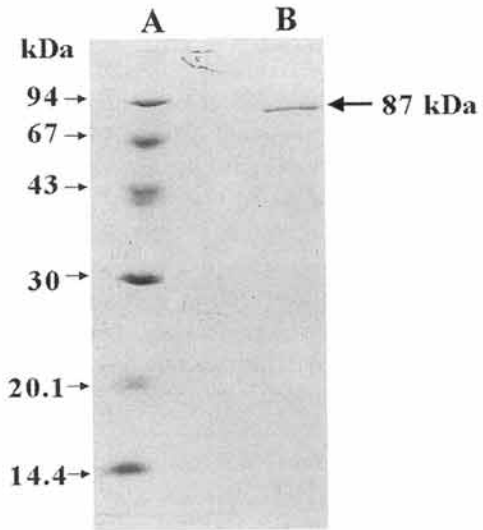

Fig. 1. SDS-PAGE of sucrose synthase from the cytosolic fraction of chickpea nodules. The purified enzyme (4 g) obtained after the Superose 6 purification step of the procedure described in Table 1 was applied to lane B.

Table 1. Purification of sucrose synthase from the cytosolic fraction of chickpea nodules.

Fraction	Activity (unit)	Protein (mg)	Specific activity (unit/mg protein)	Recovery (%)	Purification (fold)
Crude extract	43.1	73.1	0.59	100	1
30-50% $(NH_4)_2SO_4$	38.5	34.1	1.13	89	2
Sephadex G-200	27.9	.5.67	4.92	65	8
Q-Sepharose	16.4	1.84	8.92	38	15
PBA-60	10.8	0.82	13.2	25	22
Superose 6	7.4	0.45	16.4	17	28

*Nodules (25 g) were extracted and sucrose synthase activity was measured as described using assay A.

The effect of pH on the activity of sucrose synthase prepared from the cytosolic fraction of chickpea nodules is shown in Fig. 2. The purified enzyme in the cleavage direction had a sharp pH profile with optimum activity at pH 6.5 (Fig. 2). In the synthesis direction, the optimum activity was obtained at pH 9.0. The properties of sucrose synthase were determined 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 chickpea nodule sucrose synthase were similar.

Typical hyperbolic kinetics to substrates were observed for sucrose synthase in sucrose cleavage and synthesis directions. When the concentration of sucrose was varied at different concentrations of UDP, an intersecting pattern of linear double reciprocal plots was investigated (Fig. 3). Replots of the slopes and the intercepts were linear and a good fit of the data was obtained with the equation describing an intersecting initial velocity pattern. The K_m values for sucrose and UDP were 29.43.9 mM and 8.0 0.9 μM, respectively. An intersecting pattern of linear double reciprocal plots was observed when the concentration of UDP-glucose was varied at the various concentrations of fructose (Fig. 4). The K_m value for UDP-glucose was 24.1±8.0 μM (Table 2). At the concentrations of fructose greater than 25 mM, a substrate inhibition of the synthesis direction occurred. For substrate specificity, hyperbolic kinetics were observed when ADP, CDP, and GDP acted as glucosyl acceptors in the cleavage reaction. The apparent K_m values for ADP, CDP, and GDP were 0.23±0.02, 0.87± 0.04, and 1.51±0.03 mM, respectively (Table 3). The values of the apparent velocity were 8.5-, 13.5- and 32-fold lower than the velocity obtained with UDP when ADP, CDP, and GDP were used as glucosyl acceptors, respectively. The constants given for ADP and CDP are apparent values as the concentration of sucrose in the assays was not saturating. Sucrose synthase in the presence of GDP as a glucosyl acceptor showed little sucrose cleavage activity compared to the presence of UDP.

Fifty percent (I_{50}) of cleavage activity of chickpea nodule sucrose synthase was inhibited by 10 mM fructose and 14 mM UDP-glucose (Table 2). Synthesis activity was strongly

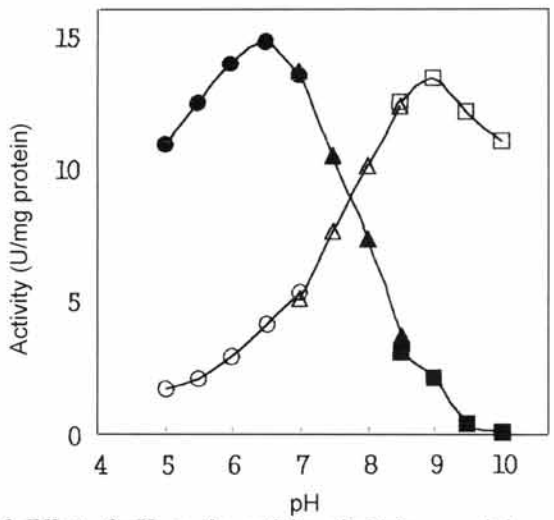

Fig. 2. Effect of pH on the activity of chickpea nodule sucrose synthase. Activities in the cleavage (●, ▲, ■) and synthesis (○, △, □) directions were determined as described using assays A and D, respectively. Buffers used were 20 mM Mes-KOH (●, ○), 20 mM Hepes-KOH (▲, △), and 20 mM Ches-KOH (■, □).

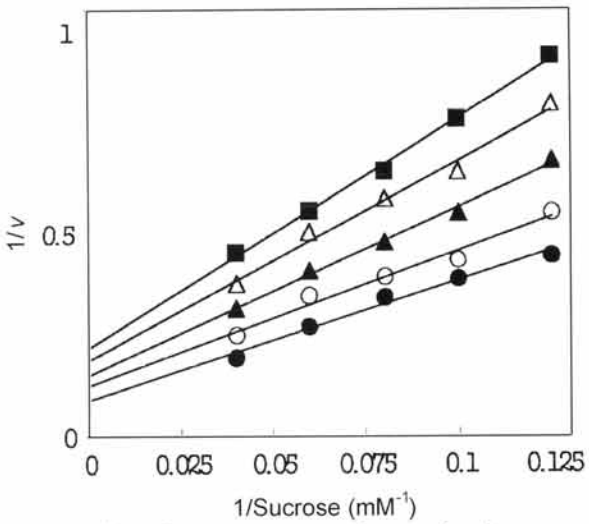

Fig. 3. The effect of sucrose concentration on the cleavage activity of sucrose synthase from the cytosolic fraction of chickpea nodules. Reaction mixtures were of the composition described for assay A except that the concentration of sucrose was varied as shown in the presence of 3 μM (●), 4 μM (○), 6.3 μM (▲), 10 μM (△), and 20 μM (■) UDP. Velocities are expressed as units/mg of protein.

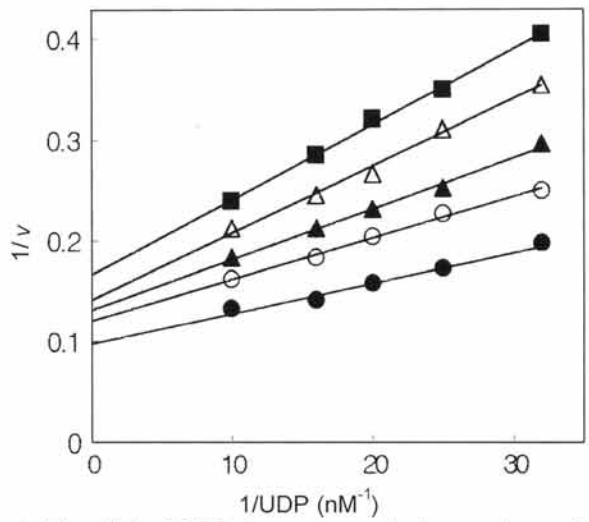

Fig. 4. The effect of UDP-glucose concentration on the synthesis activity of sucrose synthase from the cytosolic fraction of chickpea nodules. Reaction mixtures were of the composition described for assay E except that the concentration of UDP-glucose was varied as shown in the presence of 2.5 mM (●), 3 mM (○), 4 mM (▲), 5 mM (△), and 8 mM (■) UDP. Velocities are expressed as units/mg of protein.

Table 2. Characterization of sucrose synthase from the cytosolic fraction of chickpea nodules.

Characteristics	Values
Native molecular mass (kDa)	356
Subunit molecular mass (kDa)	87
	Cleavage reaction[a]
v (U/mg protein)	14.7±2.5
Optimal pH	6.5
K_m Sucrose (mM)	29.4±3.9
K_m UDP (μM)	8.0±0.9
I_{50}[b] for Fructose (mM)	10.0
I_{50} for UDP-glucose (mM)	14.0
I_{50} for Glucose (mM)	5.4
	Synthesis reaction[c]
v (U/mg protein)	13.8±2.1
Optimal pH	9.0
K_m Fructose (mM)	5.4±0.4
K_m UDP-glucose (μM)	24.1±8.0
K_m ADP-glucose (mM)	2.6±0.3
I_{50} for UDP (mM)	0.5

[a]Activity was determined as described using assay A.

[b]I_{50}, Concentration for 50% inhibition.

[c]Activity was determined as described using assay E for synthesis reaction. The kinetics parameters were obtained by fitting the data of Fig. 3 and Fig. 4 to equation 1 using the method of Cleland.[19]

Table 3. Nucleotide specificity of sucrose synthase from the cytosolic fraction of chickpea nodules.

Nucleotide	Apparent K_m (mM)	Apparent V_{max} (U/mg protein)
ADP	0.23 0.02	1.72 0.02
CDP	0.87 0.04	1.09 0.04
GDP	1.51 0.03	0.46 0.04

*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.[20]

inhibited by UDP with inhibition of 50% being given by 0.5 mM UDP. Chickpea nodule sucrose synthase was also inhibited by glucose. In the cleavage direction, 25% and 50% of activities were inhibited by glucose concentration of 3.1 and 5.4 mM, respectively. In the synthesis reaction, the enzyme followed hyperbolic kinetics when ADP-glucose acted as the glucosyl donors. Value of 2.6±0.3 mM was determined for the apparent K_m. There was no effect on the cleavage activity by UTP (5 mM), ATP (5 mM), DTT (5 mM), GSH (5 mM), 2-mercaptoethanol (5 mM), allantoin (5 mM), and EDTA (50 mM). Furthermore, the following metabolites showed 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.

Monovalent ions, such as Na^+, K^+, and NH_4^+ (final concentration, 50 mM), had no effect on the cleavage or synthesis reactions of chickpea nodule sucrose synthase. Anions, such as Cl^-, F^-, NO_3^-, SO_4^{2-}, borate, and citrate,

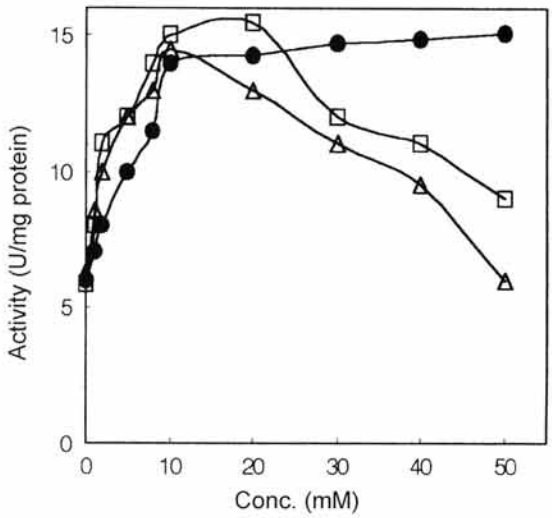

Fig. 5. Effect of divalent cations on the synthesis activity of sucrose synthase from the cytosolic fraction of chickpea nodules. Activity was determined using assay D in the presence of $MgCl_2$ (●), $CaCl_2$ (△), and $MnCl_2$ (□) in the reaction mixtures.

showed no effect on the enzyme activity at the concentration upto 50 mM in either reaction direction. Great stimulatory effects on sucrose synthesis activity were observed in the presence of Ca^{2+}, Mg^{2+}, and Mn^{2+} (Fig. 5). The maximum stimulations of activity, given by 20 mM $CaCl_2$, 10 mM $MgCl_2$ and 10 mM $MnCl_2$, were approximately 2.7-, 2.4- and 2.3-fold, respectively. Increasing the concentration of $MgCl_2$ to 50 mM $MgCl_2$ led to a small increase in activity. Ca^+ and Mn^+ inhibited the synthesis reaction, given by $CaCl_2$ concentrations above 20 mM and $MnCl_2$ concentrations above 30 mM (Fig. 5). Chickpea nodule sucrose synthase was also inhibited by $HgCl_2$ and $CuSO_4$. In the cleavage reaction, 50% of activity was inhibited by 18 μM $HgCl_2$ or 24 μM $CuSO_4$.

Discussion

Chickpea nodules contained a single form of sucrose synthase. A purification of only 28-fold yielded a preparation of sucrose synthase which was close to homogeneity. It is likely that the enzyme was from cytosolic fraction of the nodules, since the activity of sucrose synthase was not detected in the bacteroids of chickpea nodules.[12] Similar results were obtained with sucrose synthase from *Daucus carota* and soybean nodule, which support the proposal that these enzymes are located in the cytoplasm.[8,9]

Highly purified enzyme from the cytosolic fraction of chickpea nodules corresponded to a protein with an apparent native molecular mass of 356 kDa. The subunit molecular weight of the purified enzyme was 87 kDa, indicating that the native enzyme is probably a tetramer. A similar pattern of chickpea nodule sucrose synthase was found in *D. carota* and soybean nodule, which had molecular mass of approximately 320 and 400 kDa, respectively.[8,9] Furthermore, sucrose synthases of *D. carota*, mung bean seedl-

ing and soybean nodule have tetrameric structures with 80, 84, and 90 kDa, respectively.[8,9,23]

Sucrose synthase of chickpea nodules displayed typical hyperbolic kinetics with substrates in the cleavage and synthesis reactions. Typical hyperbolic kinetics have been displayed by a number of other sucrose synthases[8,9], although sigmoidal kinetics have been reported for the enzyme from sweet potato and rice grains.[24,25] Maximum activity was observed in the presence of 20 mM $CaCl_2$, 10 mM $MgCl_2$, and 10 mM $MnCl_2$ on both the cleavage and synthesis reactions. The stimulation of sucrose synthesis by divalent cations has been reported for other sucrose synthases.[8,9,23] Heavy metals were potent inhibitors of the cleavage and synthesis reactions, suggesting the involvement of a sulfhydryl group in catalysis.

Sucrose synthase from the cytosolic fraction of chickpea nodules had a high affinity for UDP. The K_m (8.0 μM) for UDP was considerably lower than the range of K_m values (0.06-8.3 mM) that had been reported for other sucrose synthases.[26,27] ADP could act as a glucosyl acceptor in the cleavage reaction but was much less effective than UDP. Sucrose synthase is thus unlikely to be significant in the formation of ADP-glucose, CDP-glucose, and GDP-glucose in chickpea nodules. Therefore, UDP is generally the preferred glucosyl acceptor for the sucrose synthase.[26,27] The affinity of sucrose synthase to UDP-glucose in chickpea nodules was similar to soybean nodule sucrose synthase but, in contrast, it was relatively high compared to those of other sucrose synthases.[26,27]

High concentrations of sucrose and low concentrations of fructose were determined in legume nodules.[28] In present study, although the reaction catalyzed by sucrose synthase is readily reversible, the purified enzyme is likely to act predominantly in the breakdown of sucrose in chickpea nodules. The higher affinity of the enzyme to UDP than to UDP-glucose, and the strong inhibition of sucrose synthase by UDP, would also favor the cleavage reaction. In the inhibition of the cleavage reaction by UDP-glucose and fructose, fine control of sucrose synthase is likely to be an important part of the metabolism for regulating the sucrose cleavage for the assimilation of fixed ammonia in the cytosolic fraction of chickpea nodules.[28]

The cytosolic fraction of chickpea nodules contains alkaline invertase and sucrose synthase to cleavage sucrose.[4,9] Both enzymes appear to be regulated mainly by the utilization of sucrose. In the cytosolic fraction of legume nodules, alkaline invertase (K_m, 10 mM) has a higher affinity to sucrose than sucrose synthase (K_m, 29.4 mM) has, and this may suggest how sucrose in the nodules is partitioned between the two reactions.[4,9] In the present study, the results indicate important functions for sucrose synthase from the cytosolic fraction of chickpea nodules in physiological process. However, further studies will be also required to investigate the molecular levels of chickpea nodule sucrose synthase, such as cDNA cloning, analysis of the cell-specific expression and alteration of its expression in legume nodules by sense/antisense transformation.

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