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CUCUMBER FRUIT SUCROSE SYNTHASE ISOZYMES*

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Key Word Index—*Cucumis sativus*; Cucurbitaceae; cucumber; sucrose synthase; isozymes; fruit.

Abstract—Two forms of sucrose synthase (SSI and SSII) were resolved from cucumber (*Cucumis sativus*) fruit pericarp and fruit peduncle tissue using DEAE-cellulose chromatography. The isozymes from peduncle tissue were characterized further. Before and after DEAE-cellulose chromatography, the apparent MW of both forms was estimated to be 540 000 using Sephadex G-200 chromatography. SSI and SSII were present in extracts of fresh peduncles homogenized in buffer containing three protease inhibitors. In the direction of UDP-glc synthesis, both enzymes from peduncle tissue had a pH optimum of 6.0. However, in the direction of sucrose synthesis, SSI and SSII had pH optima of 9.0 and 7.5, respectively. The apparent K_m values for UDP-glc and -fru were 0.34 mM and 11.5 mM, respectively, for SSI and 0.38 mM and 5.9 mM, respectively, for SSII. Both enzymes were inhibited to similar extents by UDP and both synthesized sucrose from ADP-glc at a rate 2 to 13% of the rate obtained when UDP-glc was used as the glycosyl donor. Peduncle SSII was stimulated to a greater extent by $MnCl_2$ than was SSI.

INTRODUCTION

The physiological role of sucrose synthase (UDP-glc:D-fructose 2-glucosyltransferase, EC 2.4.1.13) is often considered to be sucrose degradation[1–4]. However, the reaction is reversible and a synthetic role for the enzyme in some plant tissues has not been ruled out[1, 5–7]. It has been suggested that sucrose cleavage and sucrose synthesis, *in vivo*, may be attributed to different forms of sucrose synthase[8–10]. Several studies have demonstrated the existence of multiple forms of sucrose synthase[10–13], but there have been no kinetic comparisons of sucrose synthase isozymes from the same tissue.

Cucumber fruit peduncles contain a pathway which converts the galactosyl moieties of stachyose, the major transport sugar, to sucrose[5,6]. However, peduncles contain no detectable sucrose phosphate synthetase activity[6]. Thus, the role of sucrose synthase may be primarily synthetic in this tissue. The objective of this work was to investigate the possible existence of sucrose synthase isozymes in cucumber fruit tissues.

RESULTS AND DISCUSSION

Although there have been several reports of sucrose synthase isozymes[10–13], most studies have not revealed the presence of multiple forms[1–4, 7, 8, 14–16]. In this study, two forms of sucrose synthase were resolved from cucumber fruit tissues using DEAE-cellulose chromatography (Fig. 1). SSI and SSII eluted at salt concentrations of 0.1 M and 0.18 M KCl, respectively, from both fruit peduncle and pericarp tissue. In peduncle tissue, SSII comprised 43% of the total activity, whereas in pericarp tissue SSII comprised only 17%. The relative abundance of SSII in peduncles may imply an important physiological role of this isozyme in cucumber peduncles, or may reflect differential stability of the isozymes during preparation from the two tissues. A similar elution profile was obtained when peduncles were homogenized in extraction buffer fortified with the following protease inhibitors: 1 mM benzamidine, 1 mM phenylmethyl-sulfonylfluoride, and 5 mM amino-*n*-caproic acid (data not shown). Thus, the two forms apparently did not result from protease action during enzyme preparation. The pooled SSI and SSII fractions from DEAE-cellulose chromatography contained activities of 3 and 1.8 mkat/mg protein, respectively, when assayed in the direction of sucrose synthesis. Overall recovery of total sucrose synthase activity was 19%.

Sucrose synthase eluted as a single peak from a Sephadex G-200 column at an elution volume which corresponded to a protein with a MW of 540 000 (data

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Abbreviations: SSI, the form of sucrose synthase which eluted at 0.1 M KCl during DEAE-cellulose chromatography; SSII, the form of sucrose synthase which eluted at 0.18 M KCl during DEAE-cellulose chromatography; UDPase, uridine diphosphatase; glc, glucose; fru, fructose; gal, galactose.

not shown). SSI and SSII were chromatographed individually on the Sephadex G-200 column after resolution using DEAE-cellulose. Both forms had the same elution volume as the single peak observed prior to DEAE-cellulose chromatography. Thus, no gross changes in MW occurred during DEAE-cellulose chromatography.

Certain kinetic aspects of SSI and SSII could be compared since no UDP-gal-4'-epimerase or phosphodiesterase activity was present in either pooled enzyme. The 50° heat-treatment resulted in an 82% loss of UDPase activity and a complete loss of UDP-gal-4'-epimerase activity while only 7% of the total sucrose synthase activity was lost. Similar ratios of SSI and SSII were obtained from peduncle tissue when the heat treatment was omitted (data not shown). Due to the presence of invertase (1.6 mkat/mg protein) in the SSII preparation and UDPase in SSI (0.18 mkat/mg protein) and SSII (0.24 mkat/mg protein) preparations, the apparent K_m values for UDP and sucrose could not be compared. However, the presence of these enzymes did not preclude kinetic comparisons of SSI and SSII in the direction of sucrose synthesis nor with pH optima determinations in the direction of UDP-glc synthesis. SSI and SSII had pH optima of 6.0 when assayed in the direction of UDP-glc synthesis (Fig. 2). However, their response to pH differed when assayed in the direction of sucrose synthesis; the pH optimum of SSI was 9.0, and the pH optimum of SSII was 7.5. Although the effect of pH on activity of SSI assayed in both directions is similar to sucrose synthases from other species, the near neutral pH optimum of SSII for sucrose synthesis is clearly different from that of SSI as well as the enzyme from other sources [4, 14–16].

The apparent K_m values of the peduncle isozymes for UDP-glc and fru were 0.34 mM and 11.5 mM, respectively, for SSI and 0.38 mM and 5.9 mM, respectively, for SSII. Woolf plots were used to estimate kinetic parameters. Best fitting lines were determined using linear regression analysis; all regression coefficients were greater than 0.95.

SSII was stimulated to a greater extent by $MnCl_2$ than was SSI (Fig. 3). In the direction of sucrose synthesis, SSI and SSII were inhibited similarly by UDP at concentrations ranging from 0.1 mM (44% inhibition) to 2 mM (93% inhibition). However, estimates of the apparent K_i values for UDP were pre-

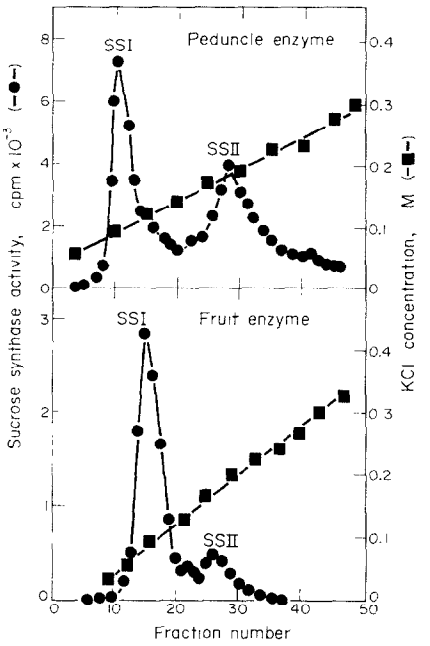

Fig. 1. Elution profile of fruit peduncle and fruit pericarp sucrose synthases from a DEAE-cellulose column (1.5 × 14 cm) previously equilibrated in 50 mM Hepes-NaOH (pH 7.2) containing 0.5 mM DTT. Peduncle sucrose synthases were eluted with a 150 ml linear gradient of 0.05 M to 0.3 M KCl in the equilibration buffer after a 75 ml wash of the column with buffer containing 0.05 M KCl. After a 75 ml wash of the column with the 50 mM Hepes-NaOH (pH 7.2) containing 0.5 mM DTT, fruit pericarp sucrose synthases were eluted with a 150 ml gradient of 0 to 0.3 M KCl in buffer. Three-ml fractions were collected at a rate of 0.55 ml/min and assayed for sucrose synthase activity in the direction of sucrose synthesis. Peduncle enzymes were pooled for further characterization: SSI, fractions 8 through 14; SSII, fractions 26 through 32.

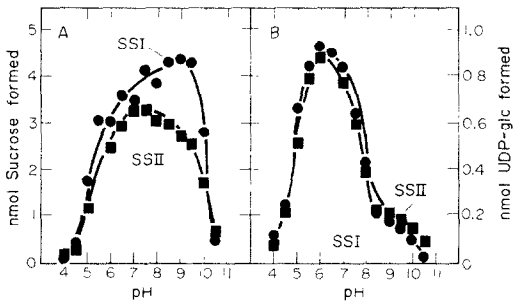

Fig. 2. The effect of pH on the activities of SSI and SSII when assayed in the direction of sucrose synthesis (A) or in the direction of UDP-glc synthesis (B). Reaction mixtures were standard except that the following buffers were used for the designated pH ranges: Na-acetate (pH 4–5), MES-NaOH (pH 5.5–6.5), Hepes-NaOH (pH 7–8), Tris-HCl (pH 8.5–9), and glycine-NaOH (pH 9.5–10.5).

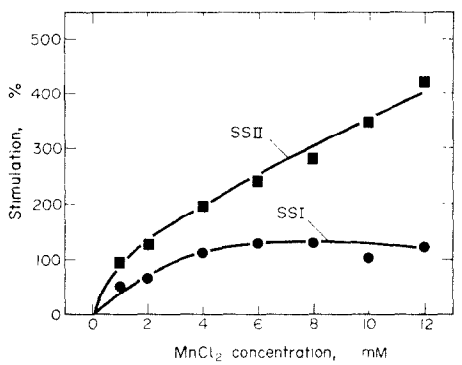

Fig. 3. The effect of $MnCl_2$ on the activities of SSI and SSII when assayed in the direction of sucrose synthesis. Reaction mixtures were as described in the Experimental except for the omission of EDTA and the use of 100 mM fru instead of 30 mM.

cluded since UDPase was present in both enzyme preparations. Under identical assay conditions, both enzymes synthesized sucrose from ADP-glc at a rate 2–13% of the rate obtained when UDP-glc was used as the glucosyl donor.

SSII activity was more abundant in peduncle tissue than in fruit pericarp tissue, was more highly stimulated by $MnCl_2$ than SSI, and had a more physiological pH optimum for sucrose synthesis (pH 7.5) than both SSI (pH 9.0) and sucrose synthases from other species [4, 14–16]. Since cucumber peduncles contain a pathway which converts the galactosyl moieties of transported stachyose to sucrose but do not contain sucrose phosphate synthetase [5, 6], it is interesting to speculate that SSII may function primarily to synthesize sucrose in cucumber peduncle tissue. Studies of additional kinetic parameters, particularly the apparent K_m values for sucrose and UDP, may be revealing in this respect. Further purification of SSI and SSII will be necessary prior to determination of these values.

EXPERIMENTAL

Plant material. Fruit peduncles were harvested from field-grown cucumber (*Cucumis sativus* L. cv Calypso) plants and stored at −70° for up to 4 months. For extraction of sucrose synthase from pericarp tissue, fruit harvested from greenhouse-grown plants were peeled and the pericarp homogenized immediately. When peduncles were homogenized in the presence of protease inhibitors, freshly harvested tissue from greenhouse-grown plants was used.

Enzyme preparation. 45 g of tissue was homogenized in 200 ml of 50 mM Hepes-NaOH (pH 7.2) containing 1 mM EDTA, 0.5 mM DTT, and 0.1% Triton X-100 using a VirTis 45 homogenizer. The extract was centrifuged at 12 000*g* for 15 min and the supernatant heated at 50° for 10 min. The heat-treated extract was re-centrifuged and the pellet discarded. Solid $(NH_4)_2SO_4$ was added to the supernatant, and the protein insoluble between 0.3 and 0.7 saturation was collected by centrifugation at 27 000*g* for 15 min and dissolved in 8 ml of 50 mM Hepes-NaOH (pH 7.2) containing 0.5 mM DTT. The extract was desalted using a Sephadex G-25 column (1.5 × 22 cm) and loaded onto a DEAE-cellulose column (Whatman DE 52, 1.5 × 14 cm). Both columns were equilibrated in 50 mM Hepes-NaOH containing 0.5 mM DTT. The DEAE-cellulose column was washed with either 75 ml of buffer or 75 ml of buffer containing 0.05 M KCl. Sucrose synthase activities were then eluted using a 150 ml linear gradient of KCl in the column buffer. The two forms of sucrose synthase routinely eluted at 0.1 M KCl (SSI) and 0.18 M KCl (SSII). The most active fractions of each form from peduncle tissue were pooled for further characterization and stored at 4°. SSI was stable for 4 months at 4°, whereas 50% of the SSII activity was lost within 3 weeks.

Gel filtration chromatography. For comparing the MW of SSI and SSII, a Sephadex G-200 column (2.5 × 50 cm) was calibrated using the following proteins: thyroglobulin, catalase, bovine serum albumin, and chymotrypsinogen A. Each protein was run separately, and its elution vol. determined by reading the *A* of each fraction at 280 nm. The column was equilibrated with 50 mM Hepes-NaOH (pH 7.2) containing 0.5 mM DTT.

Enzyme assays. Invertase was assayed using the procedure of ref. [17] which involved the detection of reducing sugars [18] formed during hydrolysis of sucrose.

UDP-gal-4′-epimerase was assayed by measuring the amount of UDP-glc formed from UDP-gal. UDP-glc was determined by measuring the formation of NADH from NAD^+ during the oxidation of UDP-glc by UDP-glc dehydrogenase [19].

UDPase activity was estimated by measuring the formation of Pi during incubation of enzyme extract with UDP under sucrose synthase assay conditions. Pi was measured colorimetrically [20].

Phosphodiesterase was assayed by measuring the formation of glc-1-P from UDP-glc. The complete reaction contained 1 μmol of UDP-glc, 5 μmol of $MgCl_2$, 1 μmol of NADP, 2 μmol of DTT, 0.5 unit of phosphoglucomutase, 0.8 unit of glc-6-P dehydrogenase, 10 μmol of Hepes-NaOH (pH 7.2), and 200 μl of enzyme extract in a total vol. of 1 ml Reactions were incubated at 25°, and the amount of NADPH formed from NADP during the oxidation of glc-6-P to 6-phosphogluconate was determined [21].

Sucrose synthase was assayed in the direction of sucrose synthesis by measuring the fru dependent formation of [^{14}C]sucrose from UDP-[^{14}C]glc. The complete reaction mixture (100 μl) contained 10 μmol of Hepes-NaOH (pH 7.2), 0.1 μmol of UDP-[^{14}C]glc (1.3 μCi/μmol), 0.1 μmol of EDTA, 0.01 μmol of DTT, 3 μmol of fru, and 20 μmol of extract. Reactions were incubated at 30° for 10 min and terminated by adding 0.4 ml of 100% EtOH. Remaining charged substrate was removed by adding 0.2 ml of Dowex 1-X8 anion exchange resin (formate form) in a 1:1 suspension with H_2O (v/v) and incubating at 25° for 20 min with constant shaking. Samples were then centrifuged for 1 min and a 0.3 ml aliquot placed into a vial containing 15 ml of toluene scintillation fluid (6 g PPO/1 and 0.3 g POPOP/1) containing 33% (v/v) Triton X-100. Radioactivity was assayed, and the fru-dependent conversion of charged substrate to neutral product was calculated. This assay was used for monitoring columns. In all experiments, sucrose synthase was assayed under conditions where sucrose formation was linear with time and proportional to the amount of enzyme extract in the reaction. The [^{14}C]sucrose product was identified by co-chromatography with authentic sucrose on paper radiochromatograms developed in a solvent of *n*-BuOH–benzene–pyridine–H_2O (5:1:3:3). Treatment of the [^{14}C]sucrose product with yeast invertase yielded only [^{14}C]glc and no [^{14}C]fru [6].

Sucrose synthase was assayed in the direction of UDP-glc synthesis by measuring the UDP-dependent formation of UDP-glc. The complete reaction (1 ml) contained 200 μmol of sucrose, 2 μmol of UDP, 200 μmol of Hepes-NaOH (pH 7.2), and 100 μl of enzyme extract. Reactions were incubated at 30° for 10 min and terminated by immersion in boiling water for 2 min. The amount of UDP-glc formed was determined by measuring the formation of NADH from NAD^+ during the oxidation of UDP-glc by UDP-glc dehydrogenase [19].

Protein determination. The amount of protein in plant extracts was estimated using the procedure of ref. [22] with casein as the standard.

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