Phytochemistry, 1978, Vol. 17, pp. 1231–1233. © Pergamon Press Ltd. Printed in England. 0031-9422/78/0801-1231$02.00/0

SUCROSE SYNTHETASE FROM TRIPLOID QUAKING ASPEN CALLUS*

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(*Revised received* 20 *January* 1978)

Key Word Index—*Populus tremuloides*; Salicaceae; quaking aspen; biochemistry; enzymes; sucrose synthetase; UDPG pyrophosphorylase.

Abstract—Sucrose synthetase and UDPG pyrophosphorylase were found in crude extracts of dark-grown triploid quaking aspen callus. The sucrose synthetase was like that from other plants, but it appeared to be membrane-bound. The activity also occurred in extracts prepared from callus of other tree species.

INTRODUCTION

There have been few reports of UDPG pyrophosphorylase (EC 2.7.7.9) and sucrose synthetase (EC 2.4.1.13) from cultured plant tissues [1–4]. The physiological role of sucrose synthetase is thought to be the formation of nucleoside diphosphate glucoses [5–10] and regulation of carbohydrate metabolism [11–14]. Since sucrose is the primary carbon source for cultured plant tissues [1, 15, 16], sucrose synthetase could provide an efficient route for utilization of this sugar.

RESULTS AND DISCUSSION

Radiochromatograms of the products from the incubation of UTP and D-glucose-[U-^{14}C]-1-phosphate (-G[^{14}C]-1-P) with fructose and crude callus extract showed that as the fructose concentration was increased, the formation of sucrose was enhanced at the expense of UDPG. These results would be compatible with the presence of sucrose synthetase and UDPG pyrophosphorylase. Fructose-6-phosphate inhibited the formation of both products; this eliminated the possibility that sucrose was formed via a sucrose phosphate synthetase. Label could also be introduced into sucrose when UTP, G-1-P, and D-fructose-[U-^{14}C] (fructose-[^{14}C]) were incubated with crude extract. Sucrose was crystallized to constant sp. act. with unlabeled sucrose. Presumed UDPG and sucrose were identical to authentic materials on PC in several solvents. Hydrolysis of UDPG and sucrose showed that the label was in a glucose residue.

Synthesis of sucrose by sucrose synthetase was also demonstrated by incubating UDPG [D-glucose-U-^{14}C] (UDPG-[^{14}C]) with fructose and crude extract. Sucrose could also be produced by incubating unlabeled UDPG and fructose-[^{14}C] with the crude extract. The reverse sucrose cleavage reaction was more difficult to demonstrate, as expected [7, 8, 10, 17]. Samples in which sucrose-[U-^{14}C] (sucrose-[^{14}C]) and UDP had been incubated with the crude extract, in which more labeled fructose than labeled glucose was found, suggested the promotion of the sucrose cleavage to form UDPG (Table 1).

Table 1. Evidence for sucrose cleavage reaction

Observation	Sample With $MgCl_2$	Sample Without $MgCl_2$	Control
Count left at origin of paper chromatograms, cpm	635	660	446
Count left on DE-81 anion exchange paper, cpm	835	883	636
Apparent UDPG count by PC, cpm	189	214	—
Apparent UDPG count by anion exchange paper binding, cpm	198	247	—

Centrifugation experiments indicated that the enzyme was membrane-bound (Table 2). A particulate sucrose synthetase was reported in lemons [18]. The average degree of purification for 5 preparations of sucrose synthetase from aspen callus was 2.9-fold with an average sp. act. of 3 nkat/mg protein. The sp. act. for purified sucrose synthetase from wheat germ was 1.3 nkat/mg

Table 2. Distribution of enzyme activity in centrifugal fractions prepared from crude extract

Preparation	Protein content (mg/ml)	Relative enzyme units per mg protein
Crude extract	1.65	100
12000 *g* supernatant	0.75	186
Redispersed 12000 *g* pellet	0.25	751
100000 *g* supernatant	1.46	59
Redispersed 100000 *g* pellet	0.23	915

* This report is taken from a dissertation submitted by L. L. Graham in partial fulfilment of the requirements for a Ph.D. degree at The Institute of Paper Chemistry.

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Table 3. Assay of cultured tree tissues for sucrose synthetase activity

Species	Age since last subculture (weeks)	Specific activity (nkat/mg protein)	(nkat/g fresh tissue)
Loblolly pine (*Pinus taeda*)	4	1.0	3.2
Loblolly pine	1.5	0.7	16.3
Douglas fir* (*Pseudotsuga menziesii*)	4	0.6	11.7
Shortleaf pine (*Pinus echinata*)	2	2.5	19.5
Western hemlock (*Tsuga heterophylla*)	2	1.4	16.2
Eucalyptus (*Eucalyptus nova-anglica*)	3.5	0.1	0.8
Triploid quaking aspen	3	1.1	10.7
Triploid quaking aspen	5.5	2.1	15.8
Triploid quaking aspen*	9	1.1	8.3

* Light-grown tissue.

protein [4], and from mung bean seedlings, 1.6 nkat/mg protein [19]. Those preparations had much higher degrees of purification, 48-fold and 252-fold, respectively. A 108-fold purified enzyme from bamboo had a sp. act. of 91.8 nkat/mg protein [14].

Several properties of the purified enzyme were determined. A single peak was observed in the ultracentrifuge sedimenting at 11.1 S in MOPS at 9°. The MW was between 350000 and 415000. Disk electrophoresis suggested a tetramer. A sharp pH optimum was found at pH 8 for sucrose synthesis. The K_m (UDPG) was 0.11 mM and K_m (fructose) was 5 mM. The Lineweaver-Burk plot indicated inhibition by excess UDPG. The effectiveness of ADPG and GDPG as substrates was lower by one and two orders of magnitude, respectively, than that of UDPG. Sucrose synthetases from other plant sources have similar properties [3, 4, 7–9, 14, 17, 19–22].

A number of other cultured tree tissues were assayed for sucrose synthetase (Table 3). Thorpe and Meier found that tobacco and carrot callus contained, respectively, 2.3 and 8.3 nkat/g fr. wt. Sp. act. (nkat/mg protein) in *crude* extracts have been reported at 1.4 for sweet potato root [9], 8.3 for ripening rice grains [7], and 0.007 for mung bean seedlings [19].

EXPERIMENTAL

Materials. All labeled substrates were purchased from the Isotope and Nuclear Division of ICN Pharmaceuticals, Inc. Whatman DE-81 anion exchange paper was purchased from Reeve Angel. Cultured tree tissues used were those being cultured at The Institute of Paper Chemistry [23, 24].

The enzyme isolation procedure was carried out at 5°. Necrotic centres were removed from dark-grown triploid aspen callus. The remaining tissue was weighed and homogenized with 1 mM dithiothreitol (DTT) in morpholinopropane sulfonate (MOPS) buffer (0.1 M, pH 8). The homogenate was centrifuged at 1000 *g*. The supernatant (crude extract) was centrifuged at 100000 *g* for 2 hr. The pellet was resuspended in DTT-MOPS and stirred for 2 hr with an equal vol of 1.5% digitonin in MOPS buffer. The digitonin-treated material was centrifuged at 1000 *g* to remove undissolved digitonin before being loaded on a Bio-Gel P-6 column and eluted with DTT-MOPS. The excluded peak was collected and treated with $(NH_4)_2SO_4$ to give a 20–60% satn ppt. The redissolved ppt was loaded on a Bio-Gel A-5 m (agarose) column which was eluted with DTT-MOPS. The fractions collected were assayed for sucrose synthetase activity in 'standard' UDPG incubation samples for 4 min. The reactions were linear for at least 5 min [24]. The most active fractions were combined to give the purified enzyme prepn used in characterization. Protein analysis was modified from refs [24, 25].

Enzyme incubation. Standard G-1-P incubation samples contained $MgCl_2$ (2 mM), UTP (0.23 mM), and 0.33 μCi G-1-P-[14C] (225 μCi/μmol) per ml of sample. Standard UDPG samples contained $MgCl_2$ (2 mM), fructose (0.1 M), unlabeled UDPG (33 μM) and 0.08 μCi UDPG-[^{14}C] (155 μCi/μmol) per ml of sample. These samples were incubated with enzyme at 30°, then frozen in an Me_2CO dry ice bath to terminate the reactions, concentrated by freeze-drying and reconstituted with measured vols of H_2O for PC or anion exchange analysis. MOPS and DTT were provided with the enzyme addition.

For PC, standards and reconstituted samples were spotted on Whatman No. 1 paper. After descending development, the resolved sample strip was cut into 2.5 cm sections that were counted by liquid scintillation. Solvent systems used were: I, *n*-BuOH–Py–H_2O (6·4·1); II, EtOAc–HOAc–H_2O (3:3·1); III, EtOAc–Py–H_2O (8:2·1); IV, *n*-BuOH EtOH–H_2O (40:11·19); V, EtOAc–HOAc–HCO_2H–H_2O (18·3·1:2), and VI, *n*-BuOH–HOAc–H_2O (4:1:5).

Anion exchange paper binding [12]. Aliquots of the reconstituted sample were spotted on disks of Whatman DE-81 paper which were washed with 30 ml H_2O and counted. The paper binds UDPG; therefore this technique was used only for samples in which UDPG-[^{14}C] was the substrate or product.

Incubation of sucrose-[^{14}C] and UDP with crude callus extract. Samples containing 4 μCi of sucrose-[^{14}C] (360 μCi/μmol) which were 0.66 mM in UDP were adjusted to pH 6. Samples (a) and (c) were also 2 mM in $MgCl_2$. Samples (a) and (b) contained extract whereas sample (c) was a control. Samples were incubated for 2 hr at 30°, freeze-dried and reconstituted.

Determination of K_m (UDPG). Samples contained 2 mM $MgCl_2$, 0.24 μCi UDPG-[^{14}C] (155 μCi/μmol), 100 mM fructose and varying concn of unlabeled UDPG. Two expts were run using different enzyme prepns. *K_m (fructose).* Because of limitations in the sp. act. of the UDPG-[^{14}C] available, it was not possible to study the effect of fructose concn at UDPG concn higher than 2 mM; however, this UDPG level was still 18 times K_m (UDPG). Samples were 2 mM in $MgCl_2$, 2 mM in UDPG, and contained 0.36 μCi of UDPG-[^{14}C] (155 μCi/μmol), varying amounts of fructose, and purified sucrose synthetase (35 μg/ml of sample). Incubations were at 30° for 4 min before freeze-drying and analysis.

Sucrose synthetase activity in other cultured tree tissues. Crude extracts were diluted with DTT-MOPS to give 0.1–0.3 mg protein per ml. The protein contents were redetermined prior to incubation in standard UDPG samples.

The MW of sucrose synthetase was estimated from its elution vol from a Bio-Gel A-5m column calibrated with ferritin, polynucleotide phosphorylase, and phenol oxidase. The values reported give the range in 4 determinations with 4 different prepns.

Acknowledgements—The authors are indebted to the pulp and paper industry for support of this research through The Institute of Paper Chemistry. Gratitude is expressed to Dr. Lawson Winton from whose laboratory the callus was obtained. The assistance of Shirley Verhagen in tissue manipulations and of John Carlson in sedimentation and electrophoretic techniques is gratefully acknowledged.

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