Involvement of sucrose synthase in sucrose synthesis during mobilization of fructans in dormant Jerusalem artichoke tubers

Giselle Martinez Noël¹, Horacio G. Pontis^{*,2}

Centro de Investigaciones Biológicas-FIBA and INBIOP-CONICET, Casilla de Correo 1348, 7600 Mar del Plata, Argentina

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Abstract

The relative contribution of sucrose synthase and sucrose-phosphate synthase to sucrose synthesis in dormant tubers of Jerusalem artichokes was determined. Feeding dormant tubers alternatively with mixtures of [¹⁴C]glucose and unlabeled fructose, and [¹⁴C]glucose and [¹⁴C]fructose has shown that sucrose synthase contributes ca. 95–97% to sucrose synthesis. This is the first report of sucrose synthesis in Jerusalem artichokes dormant tubers. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Besides starch, fructans are the most widespread reserve polysaccharides in higher plants. They are found in approximately 15% of angiosperms species as well as in several green algae and cyanobacteria [1]. Due to their economic and agricultural importance fructans have been intensively studied especially in Jerusalem artichokes, a prominent member of the Compositae family [2]. The fructans in Jerusalem artichokes belongs to the inulin series, characterized by its β (2-1) linked polyfructosylsucrose and a degree of polymerization (DP) of up to 30–40. Fructan synthesis and accumulation in Jerusalem artichokes takes place throughout the late summer and early autumn months and is mostly confined to the underground tubers. In the subsequent dormant period hydrolysis and depolymerization of fructans takes place. These events are catalyzed by the combined action of

fructan hydrolase (FH) and fructan–fructan fructosyl transferase (FFT) and results in a marked increase in concentration of small polymers of DP 3–6 [3]. During polymer hydrolysis fructose is produced, however, practically no free fructose is found in dormant tubers. Furthermore, during dormancy, the total hexose content remains fairly constant whereas the amount of combined fructose decreases from 95 to 82%, and combined glucose increases from 5 to ca. 18% [4]. These two facts may be explained by the synthesis of sucrose from fructan hydrolytic products and the subsequent synthesis of low DP fructans by FFT from sucrose. However, as far as the authors know, there has been no demonstration that sucrose synthesis occurs during tuber dormancy.

Sucrose synthesis may be catalyzed by two enzymes: sucrose-phosphate synthase (SPS) (UDP-glucose: D-fructose-6-phosphate-2-glucosyl transferase, EC 2.4.1.14) with its associate sucrose-phosphate phosphatase (SPP; sucrose-6F-phosphate phosphohydrolase, EC 3.1.3.24) and sucrose synthase (SS; UDP-glucose: D-fructose-2-glucosyl transferase, EC 2.2.1.13) [5]. While it is accepted that sucrose synthesis in photosynthetic and non-photosynthetic tissues is the product of the con-

* Corresponding author. Tel.: + 54-223-4748784; fax: + 54-223-4757120.

E-mail address: fibamdq@infovia.com.ar (H.G. Pontis).

¹ Fellow of Fundación para Investigaciones Biológicas Aplicadas.

² Career Investigator of the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

certed action of SPS and SPP [6], the role of SS is yet not fully agreed [7]. Physiological studies and kinetic analysis have strongly suggested that in vivo, SS plays a dominant role in sucrose cleavage [8]. However, recent studies have demonstrated that in developing potato tubers and other plant tissues, SS activity is reversible in vivo and can produce a fair amount (30–80%) of the newly synthesized sucrose [9].

In this report, we present evidence demonstrating the synthesis of sucrose in dormant tubers of Jerusalem artichoke. Furthermore, the data also demonstrate that synthesis of sucrose is catalyzed mainly through the action of SS.

2. Materials and methods

Jerusalem artichoke (*Helianthus tuberosus*) plants were grown in a field at Tres Arroyos, Province of Buenos Aires. Tubers were collected at the end of April (just after the aerial part has died), and kept in plastic bags at 4°C for the duration of the experiments.

For in vivo metabolic studies, a narrow cylinder (1–2 mm diameter) was bored in the middle of the tuber at right angles to the longitudinal axis using a metal hypodermic needle according to Dixon and ap Rees [10]. The channel was filled with 15–20 µl of a solution containing either 0.5 mM [^{14}C]glucose (7 MBq µmol⁻¹), and 0.5 mM unlabeled fructose, or 0.5 mM of each glucose and fructose both at a radiological concentration of 3.5 MBq µmol⁻¹. The ends of the hole were resealed with Vaseline and the tuber, covered with wet tissue paper, incubated at 4°C for 15 min or 2 h. At the end of the incubation period, a concentric cylinder of 0.8 cm diameter was cut around the hole using a cork borer and the tissue immediately frozen in liquid nitrogen. The frozen cylinder was ground to a powder and extracted three times with 95% boiling ethanol. The extracts were dried down by rotary evaporation at 30°C and taken up in 0.2 ml H₂O. Sucrose, glucose and fructose were separated by paper chromatography, developing with butanol, pyridine and water (6:4:3 by vol.). In parallel strips, sucrose, glucose and fructose standards were run and their position ascertained by developing with a silver alkaline reagent. Radioactivity was measured by cutting the experimental strip in 1-cm width segments and determined by

scintillation spectroscopy. Labeled sucrose was incubated for 1 h at 30°C in the presence of invertase and 50 mM acetate buffer pH 4.5. At the end of the incubation period, the resulting solution was reappplied to a paper chromatogram, redeveloped using phenol-water (500:125 w/w) and the label in glucose and fructose determined as indicated above.

Extracts for enzyme determination were prepared by grinding frozen tissue (liquid N₂) in a buffered solution containing 100 mM HEPES buffer pH 7.5, 1 mM EDTA, 1 mM EGTA, 20 mM β-mercaptoethanol, 0.5 mM phenylmethylsulphonyl fluoride, 0.01% triton and 20 mM MgCl₂. The extract was filtered through two layers of cheesecloth and centrifuged at 20 000 × g for 10 min. SS and SPS were assayed using methods already described by this laboratory [11,12]. Sucrose, fructose, sucrose–sucrose fructosyltransferase (SST) and FH were determined as in Santoiani et al. [13]; glucose, fructose-6-phosphate (F-6-P), glucose-6-phosphate (Glc-6-P) and hexokinase (HK) as in Pontis et al. [14]; UDP-Glc and UDP-Glc pyrophosphorylase (UGPase) as in Calderon and Pontis [15]; UDP as in Salerno and Pontis [16]; fructose-6-phosphate phosphatase (F-6-Ptase) as in Leloir and Cardini [17]; nucleoside diphospho kinase as in Bergmeyer [18] and FFT as in Pontis [19].

The results presented are means of at least three different experiments.

3. Results and discussion

In dormant tubers of Jerusalem artichoke not only the enzymes of fructan metabolism have been found, but the presence of the various enzymes of the sucrose synthetic pathway have been also demonstrated [3]. Thus, tubers appear to have the capacity to convert free hexoses to sucrose at all times from their initiation to sprouting.

The levels of metabolites (Table 1) and the activities of enzymes (Table 2) of fructan and sucrose metabolisms presented correspond to early and late dormancy stages. The metabolites level changed very slightly throughout dormancy with the exception of that of sucrose that increased continuously reaching a level about five- to six-fold higher at the end. The activities of the enzymes of fructan metabolism also varied during

Table 1
Levels of metabolites related to sucrose metabolism during dormancy^a

Metabolite	Metabolite content, $\mu\text{mol (gFW)}^{-1}$ (dormancy)	
	Early (May)	Late (September)
Fructose	0.26 ± 0.02	0.61 ± 0.04
Glucose	0.05 ± 0.01	0.06 ± 0.008
Sucrose	12.0 ± 0.8	72.0 ± 5.7
F-6-P	0.05 ± 0.003	0.06 ± 0.004
Glc-6-P	0.06 ± 0.007	0.08 ± 0.009
UDP-Glc	0.44 ± 0.04	0.60 ± 0.05
UDP	0.06 ± 0.009	0.07 ± 0.008

^a The results are the means $\pm$ S.E. of three individual tubers.

Table 2
Activities of enzymes of sucrose and fructan metabolisms during dormancy^a

Enzyme	Enzyme activity, $\mu\text{mol product (gFW)}^{-1} \text{ h}^{-1}$ (dormancy)	
	Early (May)	Late (September)
SS	9.2 ± 0.45	12.6 ± 1.02
SPS	0.51 ± 0.09	0.98 ± 0.12
UGPase	580 ± 46	608 ± 52
HK	0.70 ± 0.06	0.80 ± 0.07
F-6-Ptase	2.4 ± 0.16	2.7 ± 0.19
NUDIKI	12.5 ± 1.82	14.7 ± 2.02
SST	n.d. ^b	n.d. ^b
FFT	0.23 ± 0.03	0.075 ± 0.05
FH	0.068 ± 0.004	0.48 ± 0.10

^a The results are the means $\pm$ S.E. of three individual tubers.

^b n.d. not detected.

Table 3
Incorporation of ^{14}C into the glucose and fructose moieties of sucrose after injecting different [^{14}C]hexoses into stored Jerusalem artichokes tubers^a

Hexose supplied	^{14}C in glucose/ ^{14}C in fructose ratio in sucrose after	
	15 min	2 h
[^{14}C]glucose and unlabeled fructose	23.0 ± 2.8	26.7 ± 3.2
[^{14}C]glucose and [^{14}C]fructose	4.3 ± 0.6	0.99 ± 0.05

^a See Section 2 for details. The results are the means $\pm$ S.E. of three individual tubers.

dormancy. As expected SST could not be detected, while FH activity increased at the end of dormancy and that of FFT decreased during it.

Comparison of the activities of SS and SPS showed that SS activity was ten- to 12-fold higher than SPS (Table 2) throughout all dormancy. The presence of SPS, which has not been reported before in Jerusalem artichoke tubers (Battaglia and Pontis, unpublished) raised the question as to which of the two enzymes (or both) catalyzes sucrose synthesis during dormancy.

To address this question, and to determine the relative contribution of SS and SPS to sucrose synthesis, labelling experiments were carried out as described by Geigenberger and Stitt [9] and Déjardin et al. [20].

The experiments conducted were based on the following hypothesis. Sucrose synthesized by SPS will be symmetrically labeled on its glucosyl and fructosyl moieties irrespective of whether [^{14}C]glucose or [^{14}C]fructose is supplied. This is due to the presence of phosphoglucosomerase which catalyses an isotopic equilibration between glucose-6-phosphate and fructose-6-phosphate [21]. On the contrary, if sucrose is synthesized via SS, the glucosyl residue will be labeled from both hexoses. However, the fructosyl moiety will now be derived directly from the free fructose pool: it will be labeled from [^{14}C]fructose, but not from [^{14}C]glucose. To distinguish the contribution of SPS and SS to sucrose synthesis we, therefore, compared the intramolecular labelling of sucrose from [^{14}C]glucose and unlabeled fructose, or from [^{14}C]glucose and [^{14}C]fructose. Since the experimental rationale depends on limiting the possible recycling of label from [^{14}C]glucose into the cold endogenous pool of fructose, we used short incubations times.

The results of Table 3 indicate that the glucosyl moiety of sucrose contained between 23- and 27-fold more label than the fructosyl moiety when only the glucose supplied was labeled, whether the incubation time was 15 min or 2 h. When both added hexoses were labeled, the amount of ^{14}C in glucosyl and fructosyl moieties of sucrose reached equilibrium after 2 h from an initial disproportion after 15 min incubation. Therefore, the contribution of SS to sucrose synthesis during the experiment can be estimated from the analysis of the intramolecular labelling pattern in sucrose as mentioned above. The relative contribution of SS was estimated to be 95–97% by the formula:

$$\%SS = \left(1 - \frac{^{14}\text{C in fructosyl moiety of sucrose}}{^{14}\text{C in glucosyl moiety of sucrose}} \right) \times 100$$

The results obtained when both added hexoses were labeled and incubated for 15 min may be taken as an indication that labeled fructose is diluted with the endogenous pool of fructose with the result that the label is not equally distributed in sucrose on short incubation time. This difference disappears on longer incubation (2 h) and also shows that a small percentage of the sucrose formed is synthesized by SPS.

Sucrose synthase has been shown to contribute to sucrose synthesis in *Ricinus cotyledons* [9], *Chenodopium rubrum* cells [9], pea seed coat [20], and developing potato tubers [9] in different degrees up to 75%. On the contrary, in stored potato tubers the major sucrose synthesis occurred via SPS [9]. In stored Jerusalem artichoke tubers, the activity of SS is relatively high (see above) compared to that of SPS. For sucrose synthase to be involved in the synthesis of sucrose during dormancy, fructose produced by fructan hydrolysis must move from the vacuole into the cytosol. The newly synthesized sucrose in turn moves into the vacuole where it serves as a preferred acceptor for FFT [22]. Thus the net result is an increase in the level of low DP members (DP 3–6) of the fructan series, as it was described by Jefford and Edelman [23] in their studies of fructan mobilization during dormancy.

These results demonstrate for the first time that sucrose synthesis does occur during dormancy in Jerusalem artichoke tubers, and furthermore, that sucrose synthase is the enzyme responsible for it. The results presented in this communication also suggest that in the appropriate metabolic environment, the main function of sucrose synthase is synthesizing sucrose. Further studies will be needed to understand the role of SPS in dormant tubers where the enzyme is active but its contribution to sucrose synthesis is minor.

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