Plant Science 178 (2010) 1–8

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Review

The Cinderella story of sucrose hydrolysis: Alkaline/neutral invertases, from cyanobacteria to unforeseen roles in plant cytosol and organelles

Walter A. Vargas [a,1], Graciela L. Salerno [a,b,*]

[a] Centro de Investigaciones Biológicas, FIBA, Mar del Plata, Argentina
[b] Centro de Estudios de Biodiversidad y Biotecnología (CEBB-MdP), CONICET, Argentina

ARTICLE INFO

Article history:
Received 3 August 2009
Received in revised form 27 September 2009
Accepted 28 September 2009
Available online 2 October 2009

Keywords:
Alkaline/neutral invertases
Subcellular localization
Sucrose metabolism
Signalling pathways

ABSTRACT

Over the past decades, considerable advances have been made in understanding the crucial role of sucrose and the regulation of its metabolism in plant life. Recent studies in cyanobacteria and the analysis of several genomic sequences point towards an ancient origin of plant sucrose metabolism before the cyanobacterial phylogenetic radiation. In agreement with the generally accepted cyanobacterial endosymbiotic origin of plant chloroplasts, most of the cyanobacterial genes were transferred to the nucleus and their protein products were preferentially re-imported to the plant organelle. In the case of sucrose metabolism, the enzymes sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP), responsible of the disaccharide synthesis, and sucrose synthase (SuS) and alkaline/neutral invertases (A/N-Inv), involved in sucrose cleavage, appear to have a cyanobacterial origin. However, whereas SPS and SPP are likely to be exclusively localized in the cytosol of modern plant cells, SuS and A/N-Inv isoforms are distributed between the cytosol and different subcellular locations. Particularly, A/N-Invs are the least studied proteins of sucrose catabolism. They were somewhat underestimated, and thought to play no relevant role in carbon metabolism. However, some striking recent findings about the presence of A/N-Inv forms inside plant organelles, as well as the description of novel physiological functions, led us to re-evaluate the importance of these Cinderella enzymes. The additional roles uncovered for A/N-Invs disclose new scenarios for the interconnection between the cytosol and organelles and for complex crosstalk signalling pathways.

Contents

1. Introduction . . . 1
1.1. Proteins involved in plant sucrose metabolism . . . 1
1.2. Location of sucrose metabolizing enzymes in the plant cell . . . 2
2. Alkaline/neutral invertases: little known enzymes with surprising locations and roles . . . 2
2.1. A/N-Inv isoforms . . . 3
2.2. A/N-Inv in the cytosol . . . 3
2.3. A/N-Inv isoforms associated with the plasma membrane and the nucleus . . . 3
2.4. A/N-Inv in chloroplasts . . . 4
2.5. A/N-Inv in mitochondria . . . 5
3. Evolution and phylogenetic relationships of A/N-Invs . . . 5
4. Conclusions and future directions . . . 6
Acknowledgements . . . 7
References . . . 7

1. Introduction

1.1. Proteins involved in plant sucrose metabolism

Sucrose (α-D-glucopyranosyl β-D-fructofuranoside), one of the most abundant products in nature, is not only the main

* Corresponding author. Tel.: +54 223 474 8257; fax: +54 223 475 7120.
E-mail address: gsalerno@fiba.org.ar (G.L. Salerno).
[1] Present address: The J. Craig Venter Institute (JCVI), Rockville, USA.

0168-9452/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2009.09.015

photosynthesis-derived compound and the predominant molecule of carbon translocation in most plants, but also plays a central role in their functional biology and in responses to environmental stresses [1]. Moreover, it has become evident that sucrose and its hydrolysis products are important metabolic signals that modulate gene expression and regulate plant development [2–5].

Sucrose metabolism has been widely studied and considerable advances have been made in the understanding of its regulation in plants [6,7]. The pathway for sucrose biosynthesis involves the sequential action of sucrose-phosphate synthase (SPS, EC 2.4.1.14) and sucrose-phosphate phosphatase (SPP, EC 3.1.3.24) yielding free sucrose and inorganic phosphate (Pi) [8]. The hydrolysis of the intermediate, sucrose-6P, leads to an essentially irreversible synthesis pathway that provides an efficient production of sucrose, even at low concentrations of substrates.

The regulation of sucrose hydrolysis has become a central issue in plant carbon metabolism. Utilization of sucrose as a source of carbon and energy depends on the breakdown of the α1–β2-glycosidic bond, either by the action of invertases that irreversibly hydrolyse the disaccharide to glucose and fructose, or sucrose synthase (SuS, EC 2.4.1.13), a glucosyltransferase that catalyzes a readily reversible reaction [6,7]. However, SuS is usually assigned a role in sucrose cleavage under most physiological conditions. In the presence of a nucleoside diphosphate (preferentially UDP, in plants) SuS leads to the production of sugar nucleotides, which can act as glucose donor in the synthesis of cellulose and callose in most plant species [6,9–11]. To complete the picture of sucrose metabolism enzymes in the plant cell, there are two classes of invertase activity, initially differentiated by their optimum pHs *in vitro*: (i) acid invertases (Ac-Invs, EC 3.2.1.26, β-fructofuranosidases) with a characteristic optimum pH between 4.5 and 5.0 and (ii) alkaline/neutral invertases (A/N-Invs) with pH optima in the range of 6.5–8.0 [12,13].

1.2. Location of sucrose metabolizing enzymes in the plant cell

The enzymes of sucrose metabolism, with the exception of Ac-Invs, were thought to be cytosolic [6,12–14]. The different localizations exhibited by Ac-Invs granted these enzymes important physiological roles and prompted an extensive and detailed study in several plant species. It is well-accepted that plant Ac-Invs are glycoproteins evolutionarily related to invertases from yeast and bacteria, and responsible for the hydrolysis of sucrose in the intercellular space (or the cell wall), and also inside vacuoles [15]. Thus, these locations reflect their involvement in various aspects of the plant life cycle, as controlling sucrose allocation and plant development, in the response of plants to environmental stimuli, cell enlargement, responses to wounding and pathogen attack [16–18].

SPS, SuS and A/N-Invs were thought to be cytosolic proteins [19]. However, recent evidence indicates that only the sucrose biosynthesis pathway via SPS and SPP is exclusively located in the cytosol of the plant cell, since sucrose catabolism by SuS and A/N-Inv isoforms may also occur in other subcellular locations. In the present article we will focus our attention mainly on the novel localizations of A/N-Invs.

The central role of SuS enzymes in controlling the mobilization of sucrose into many important metabolic pathways prompted the investigation of the different isoforms present in a plant species [20–24]. Although SuS activity was initially described in the soluble protein fraction, more recent reports demonstrated that after phosphorylation, a significant pool of the protein is membrane-bound in association with the cellulose/callose synthase complex, supplying substrates for cell-wall biosynthesis [9,25,26]. SuS is also associated with the tonoplast in *Beta vulgaris* cells, corroborating its involvement in sucrose mobilization from the vacuole in sucrose-storing organs [27]. In sycamore (*Acer pseudoplatanus*) cells, SuS was demonstrated to be simultaneously targeted to plasmalemma and tonoplast membranes [28]. Interestingly, there was a lack of direct relationship between membrane type location and degree of phosphorylation, but the data supported the relevance of phosphorylation to SuS activity [28]. In contrast, in maize plants, the phosphorylation state of SuS does play a crucial role in anchoring the protein to the cell membranes. Two phosphorylated forms of the enzyme (SUS1 and SUS-SH1) are associated with membranes, whereas a non-phosphorylated form (SUS2) localized to the cytosol [29]. Association of SuS with the Golgi fraction has also been described in maize [30] and linked to the synthesis of cellulose polymers. Similarly, in tobacco pollen tubes, SuS isoforms occur in the soluble, plasma membrane and Golgi fractions, as well as a SuS-like protein seems to be in association with the cell wall [31]. In pollen tubes, a phosphorylated SuS form is more abundant in the cytoplasm and associated with the cell wall, and a non-phosphorylated protein is specific to the plasma membrane [31]. The three maize SuS protein sequences revealed that the SUS-SH1 protein is marked by a putative mitochondrial targeting signal at its N-terminus [32], and the SUS1 and SUS-SH1 isoforms are partly localized in mitochondria and nuclei [32,33]. *Arabidopsis thaliana* has a six-member SuS gene family, whose protein products are closely related to each other in both sequence and general kinetic properties. These genes have different spatial and temporal patterns of expression [34]. The *Arabidopsis* SUS2 seed isoform seems to be mainly localized in the plastids of the embryo [35]. Remarkably, despite the accepted importance of SuS isoforms, it was recently reported that none of the six isoforms in *Arabidopsis* is individually required for normal growth and reproduction [36]. In addition, data from a comprehensive loss-of-function study revealed that SuS isoforms are not required for cellulose synthesis in *Arabidopsis*, and that SUS6, and probably SUS5 are confined to phloem sieve elements, and are involved in callose synthesis in sieve plates [36]. These led to new questions about the precise role of sucrose catabolism by different SuS isoforms in plants.

On the other hand, where are A/N-Invs localized? These elusive enzymes have been barely studied in the past because of their low and unstable activity. They were described as cytosolic proteins, and somewhat underestimated, and thought to play no relevant role in carbon metabolism and plant development [16,37–43]. Since A/N-Invs are the least studied proteins of sucrose catabolism, their physiological function remained largely unknown. Some striking recent findings about the presence of functional A/N-Inv forms inside plant organelles, as well as the description of novel physiological roles, necessitate a re-evaluation of the importance of these enzymes and have revived interest in their investigation. The aim of the present review is to discuss the recently reported findings on A/N-Invs that suggest these Cinderella enzymes have novel cytosol-organelle metabolic connections and previously unforeseen roles in plant development.

2. Alkaline/neutral invertases: little known enzymes with surprising locations and roles

A/N-Invs are a group of intriguing enzymes found in oxygenic photosynthetic organisms [13,44]. Compared with the other sucrose metabolism enzymes, A/N-Invs have been "kept in the cinders", hardly taken into account in biochemical, physiological and molecular studies. Because of their generally low and labile activity, biochemical studies and purification to homogeneity of these enzymes resulted rather difficult compared with other sucrose metabolism proteins. In contrast to Ac-Invs, A/N-Invs are not glycosylated and do not belong to the β-fructofuranosidase family since they hydrolyze sucrose but not other β-fructose-containing sugars [13,41,44,45]. A/N-Inv activity was shown to be