PERGAMON

Phytochemistry 60 (2002) 437–440

PHYTOCHEMISTRY

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Molecules of Interest

The function of trehalose biosynthesis in plants

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Received 16 April 2002

Abstract

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) occurs in a large variety of organisms, ranging from bacteria to invertebrate animals, where it serves as an energy source or stress protectant. Until recently, only few plant species, mainly desiccation-tolerant 'resurrection' plants, were considered to synthesise trehalose. Instead of trehalose, most other plants species accumulate sucrose as major transport sugar and during stress. The ability to synthesize sucrose has probably evolved from the cyanobacterial ancestors of plastids and may be linked to photosynthetic function. Although most plant species do not appear to accumulate easily detectable amounts of trehalose, the discovery of genes for trehalose biosynthesis in *Arabidopsis* and in a range of crop plants suggests that the ability to synthesise trehalose is widely distributed in the plant kingdom. The apparent lack of trehalose accumulation in these plants is probably due to the presence of trehalase activity. After inhibition of trehalase, trehalose synthesis can be detected in Arabidopsis. Since trehalose induces metabolic changes, such as an accumulation of storage carbohydrates, rapid degradation of trehalose may be required to prevent detrimental effects of trehalose on the regulation of plant metabolism. In addition, the precursor of trehalose, trehalose-6-phosphate, is probably involved in the regulation of developmental and metabolic processes in plants. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Arabidopsis thaliana*; Trehalose; Trehalose-6-phosphate synthase; Trehalose-6-phosphate phosphatase; Trehalase; Stress protection; Sugar signalling; Cruciferae

1. Introduction

Trehalose (Fig. 1) is a non-reducing disaccharide that occurs in a large range of organisms, such as bacteria, fungi, nematodes and crustaceans. In addition to its function as a storage carbohydrate and transport sugar, trehalose plays an important role in stress protection, especially during heat stress and dehydration (Wiemken, 1990; Crowe et al., 1998). Despite the wide distribution of trehalose formation, trehalose had until recently only been found in a few plant species, whereas the majority of plants were not considered to contain trehalose (Müller et al., 1995a).

However, functional genes encoding enzymes of trehalose synthesis, i.e. trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) (Fig. 2), have been identified in *Arabidopsis* (Blázquez et al., 1998; Vogel et al., 1998). TPS and TPP homologues have also been found in e.g. rice, soybean and tomato. Given the apparent lack of trehalose accumulation, the question arises whether these plant species really synthesise trehalose and why they do not use trehalose as a stress protectant.

2. Protective properties of the trehalose molecule

Trehalose has been shown to stabilise proteins and membranes under stress conditions, especially during desiccation. By replacing water through hydrogen bonding to polar residues, trehalose prevents the denaturation of proteins and the fusion of membranes. In addition, trehalose forms glasses (vitrification) in the dry state, a process that may be required for the stabilisation of dry macromolecules (Crowe et al., 1998). Furthermore, trehalose remains stable at elevated temperatures and at low pH and does not undergo Maillard browning with proteins. These protective properties of trehalose are clearly superior to those of other sugars, such as sucrose, making trehalose an ideal stress protectant.

In accordance with the proposed function of trehalose in stress protection, accumulation of trehalose has been demonstrated for a few desiccation-tolerant 'resurrection'

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PII: S0031-9422(02)00137-1

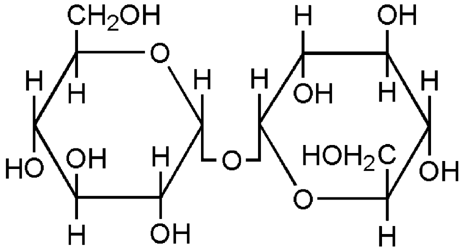

Fig. 1. Structure of trehalose.

plants, including the clubmosses *Selaginella lepidophylla* (Zentella et al., 1999) and *S. sartorii* (Iturriaga et al., 2000) and the angiosperm *Myrothamnus flabellifolius* (Müller et al., 1995a).

3. Is trehalose synthesis common in higher plants?

Early studies reporting the occurrence of trehalose in plants have to be treated with care. In some cases, the techniques used for the identification of trehalose lacked selectivity; in other cases, the presence of trehalose may have been caused by microbial contamination (Müller et al., 1995a). It has become clear that most plant species do not accumulate easily detectable amounts of trehalose. The discovery of *Arabidopsis* genes with homology to TPS and TPP genes from other organisms was therefore surprising. Yeast complementation studies have shown that Arabidopsis has at least two functional genes encoding TPP (*TPPA* and *TPPB*; Vogel et al., 1998) and one gene encoding TPS (*TPS1*; Blázquez et al., 1998). Overall, 11 TPS-like genes have been found in *Arabidopsis* (Leyman et al., 2001), some of which encode proteins with a TPP-like domain in addition to the TPS-like domain (Goddijn and van Dun, 1999). The function of these bipartite proteins is, however, not known (Vogel et al., 2001).

Although the *Arabidopsis* TPP and TPS genes have been demonstrated to be expressed in all tested organs (Blázquez et al., 1998; Vogel et al., 1998, 2001; Eastmond et al., 2002), trehalose contents in *Arabidopsis* are close to the detection limit (<1 mg g^{-1} DW; Müller et al., 2001). This apparent lack of trehalose accumulation is probably due to the activity of an *Arabidopsis* trehalase (Fig. 2). After inhibition of trehalase activity by addition of the trehalase inhibitor validamycin A to the growth medium, the content of trehalose in sterilely grown *Arabidopsis* plants did indeed increase to easily detectable amounts (to about a sixth of the sucrose content; Vogel et al., 2001). The identity of trehalose in these *Arabidopsis* plants was confirmed by GC–MS analysis. Metabolic profiling using GC–MS analysis has also led to the identification of trehalose in potato (Roessner et al., 2000). These findings suggest that the ability to synthesise trehalose is a common phenomenon in higher plants.

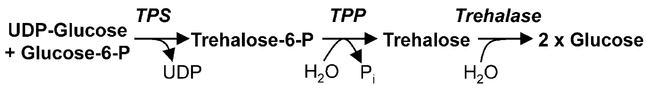

Fig. 2. Trehalose metabolism in plants; TPS = trehalose-6-phosphate synthase; TPP = trehalose-6-phosphate phosphatase.

4. The role of trehalose biosynthesis in the regulation of carbon metabolism

It is unlikely that trehalose contents in plants—other than resurrection plants—are high enough to be directly involved in stress protection. The observation that most of the trehalose formed in *Arabidopsis* is simultaneously being degraded by trehalase raises the questions of the function of trehalose biosynthesis. In yeast, the trehalose biosynthetic pathway plays an important role in the regulation of carbon metabolism: The precursor of trehalose, trehalose-6-phosphate (T6P), prevents an uncontrolled influx of glucose into glycolysis (Thevelein and Hohmann, 1995). This effect can, at least in part, be explained by an inhibition of hexokinase activity by T6P (Blázquez et al., 1993). Since hexokinase acts as a sugar sensor in yeast and probably also plants, it was suggested that T6P may be involved in the regulation of plant metabolism (Goddijn and Smeekens, 1998). This view was recently supported in a study by Eastmond et al. (2002) who reported that an *Arabidopsis* mutant with an insertion in the *TPS1* gene is impaired in embryo maturation in the phase of storage reserve accumulation (Table 1). In contrast to yeast, T6P does, however, not inhibit the activity of hexokinase (Eastmond et al., 2002). The synthesis of T6P may also play a role in the regulation of photosynthetic carbon metabolism: Transgenic tobacco plants expressing the *Escherichia coli* TPS gene exhibit enhanced rates of photosynthesis per unit leaf area, whereas photosynthesis is reduced in plants expressing the *E. coli* TPP gene (Paul et al., 2001). Furthermore, transgenic plants expressing the *E. coli* or yeast TPS genes show a variety of other phenotypic effects, including stunted growth and an inhibition of leaf expansion (Goddijn et al., 1997), suggesting additional developmental functions of T6P formation.

While the precise mechanisms of T6P action remain largely unresolved, possible targets of trehalose itself in the regulation of carbon metabolism have been identified (Table 1). Similar to sucrose, trehalose induces enzymes involved in the accumulation of storage carbohydrates in photosynthetic tissues. In barley, externally supplied trehalose induces the activity of sucrose:fructan-6-fructosyltransferase, an enzyme of fructan biosynthesis (Müller et al., 2000). In *Arabidopsis*, trehalose strongly induces the expression of *ApL3*, a gene encoding a large subunit of ADP-glucose pyrophosphorylase, which is an important enzyme in starch biosynthesis. This induction of *ApL3*

Table 1
Evidence for a role of trehalose biosynthesis in the regulation of carbon metabolism in plants

Active molecule	Regulated process/pathway	Method	Reference
Trehalose-6-P	Sugar accumulation during drought stress	Transgenic tobacco plants expressing the *E. coli* TPS gene	Pilon-Smits et al. (1998)
Trehalose-6-P	Photosynthetic capacity	Transgenic tobacco plants expressing the *E. coli* TPS or TPP genes	Paul et al. (2001)
Trehalose-6-P	Embryo maturation	*Arabidopsis* mutant with disruption in *TPS1*	Eastmond et al. (2002)
Trehalose	Fructan biosynthesis in leaves	Feeding of trehalose to barley leaves	Müller et al. (2000)
Trehalose	Carbohydrate contents in roots and nodules	Treatment of soybean plants with the trehalase inhibitor validamycin A	Müller et al. (1995b)
Trehalose	Sucrose metabolism in roots	Feeding of trehalose to soybean plants	Müller et al. (1998)
Trehalose	Starch biosynthesis in cotyledons and leaves	Feeding of trehalose to Arabidopsis seedlings	Wingler et al. (2000)
Trehalose	Starch biosynthesis in cotyledons and leaves	Complementation of starch biosynthetic mutants by trehalose feeding	Fritzius et al. (2001)

expression leads to increased ADP-glucose pyrophosphorylase activity, an overaccumulation of starch in the shoots and decreased root growth (Wingler et al., 2000; Fritzius et al., 2001).

So far, it is not clear to what extent endogenously formed trehalose is involved in the regulation of metabolism. It is possible that trehalase activity normally keeps cellular trehalose concentrations low in order to prevent detrimental effects of trehalose accumulation on the regulation of carbon metabolism. Such a role of trehalase may be of particular importance in interactions of plants with trehalose-producing micro-organisms. In support of this hypothesis, expression of the *Arabidopsis* trehalase gene and trehalase activity were found to be strongly induced by infection of *Arabidopsis* plants with the trehalose-producing pathogen *Plasmodiophora brassicae* (Brodmann et al., 2002).

5. Why do most plants accumulate sucrose and not trehalose?

Apart from plants, only cyanobacteria have been found to be capable of sucrose synthesis (Porchia and Salerno, 1996). It is therefore feasible that the ability to form sucrose is derived from the cyanobacterial ancestors of plastids and that sucrose synthesis is linked to photosynthetic function. Due to its higher solubility, especially at low temperatures, sucrose may also be more suited than trehalose as a transport sugar at high phloem concentrations (around 1 M). In addition, sucrose transport is energetically superior to trehalose transport (Wiemken, 1990). Due to its high free energy of hydrolysis, sucrose can be cleaved not only by invertase into glucose and fructose, but also by sucrose synthase into UDP-glucose and fructose, thereby preserving part of the energy. Even during stress, trehalose accumulation may not always be advantageous. Leyman et al. (2001) suggested that plants accumulate other protective compounds than trehalose, because high trehalose concentrations are incompatible with chaperone-assisted protein re-folding during stress recovery. A further advantage of sucrose over trehalose may be its reduced availability for microbial metabolism in plant-microbe interactions. While some microbial pathogens and symbionts are capable of sucrose utilisation, others, e.g. ectomycorrhizal fungi, typically lack invertase activity.

Instead of accumulating large amounts of trehalose, plants appear to have evolved a sophisticated system for the synthesis and breakdown of T6P and trehalose. Tight control of the cellular concentrations, would allow a role of T6P and trehalose in the regulation of plant metabolism and development. More research is required to study the enzymatic function and the expression of individual members of the TPS gene family. In addition, a more detailed analysis of T6P and trehalose contents is necessary to understand the role of these metabolites in embryo development and in photosynthetic metabolism. Work with *Arabidopsis* mutants disrupted in genes of trehalose metabolism will help shed new light on the function of trehalose synthesis in plants.

Acknowledgements

I am indebted to Andres Wiemken and Thomas Boller for giving me the opportunity to work on trehalose metabolism in their laboratory. I would also like to thank Matthew Paul for critical reading of the manuscript.

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