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ANALYSIS OF TREHALOSE IN *ARABIDOPSIS THALIANA* L. IN ADDITION, HELPFUL AT ALL STAGES OF HD

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ABSTRACT

Trehalose is a disaccharide that occurs naturally in insects, plants, fungi, and bacteria. The major dietary source is mushrooms. Trehalose is used in bakery goods, beverages, confectionery, fruit jam, breakfast cereals, rice, and noodles as a texturizer, stabilizer, humectant, or formulation aid with a low sweetening intensity. Trehalose is a naturally occurring disaccharide with known protein and membrane stabilizing capability. Although trehalose absorption in humans has not been well studied, a small fraction (0.5%) is likely to be absorbed by passive diffusion, as has been demonstrated for other dissacharides [26]. In mammalian cell culture, trehalose is moved from the extracellular to intracellular compartment via a fluid phase endocytotic mechanism, and is dependent on extracellular concentration [11]. Because of these unique chemical properties, this molecule has been the focus of study in several neurodegenerative diseases, which are associated with the misfolding of disease-specific proteins. Arabidopsis thaliana is a small flowering plant that is widely used as a model organism in plant biology. Arabidopsis is a member of the mustard (Brassicaceae) family, which includes cultivated species such as cabbage and radish. Arabidopsis is not of major agronomic significance, but it offers important advantages for basic research in genetics and molecular biology. In this study, one Arabidopsis thaliana L. in the stress conditions, growthing was analysed for the presence of trehalose. Using as anion-exchange high performance liquid chromatography (HPLC) analysis, trehalose was highest in flower and root. Furthermore, trehalose metabolizing enzymes, trehalose-6-phosphate synthase (TPS) and trehalase enzyme activities were measured in flower and root. TPS activity sharply increased under stress conditions growth.

Keywords: HPLC, Analysis, Trehalose, Humans, Arabidopsis thaliana L.

INTRODUCTION

Trehalose: Sweet Rescue

Trehalose is a naturally occurring disaccharide with known protein and membrane stabilizing capability.Because of these unique chemical properties, this molecule has been the focus of study in several neurodegenerative diseases, which are associated with the misfolding of disease-specific proteins.These conditions include Alzheimer"s disease(AD) an amyloid proteinopathy,Huntington"s disease (HD), an expanded polyglutamine proteinopathy, and oculopharyngeal muscular dystrophy (OPMD), an expanded polyalanine proteinopathy. In each disease, specific misfolded aggregate-prone proteins are resistant to the normal cellular processes of protein turnover and accumulate in insoluble inclusions in regions specific to each disease. While insoluble aggregates correlate with disease progression, there is increasing evidence that the initiating and most toxic events are caused by soluble protein oligimers or microaggregates. Trehalose is thought to work by interfering with production or enhancing destruction of toxic fragments.

Natural Functions of Trehalose

One of the fascinating aspects of trehalose is its presence in various organisms that can survive at the extremes of temperature and dehydration. This observation led to work which showed that trehalose is a naturally occurring reducer of cell stress, protecting these organisms from extremes in heat shock

and osmotic stress [7]. Trehalose is thought to act by altering or replacing the water shell that surrounds lipid and protein macromolecules [4]. It is thought that its flexible glycosidic bond allows trehalose to conform to the irregular polar groups of marcromolecules. In so doing, it is able to maintain the three-dimensional structure of these biologic molecules under stress, preserving biologic function.

Therapeutic Uses

As an extension of its natural capability to protect biological structures, trehalose has been used for the preservation and protection of biologic materials. It stabilizes bioactive soluble proteins such as monoclonal antibodies and enzymes for medical use [5]. It stabilizes proteins for inhaled use [23]. It is used to preserve cellular blood products for transfusion and greatly extends the shelf life of platelets [6], and cord blood [31]. It is used to preserve embryos during freeze-drying where it increases viability [24]. It is used in cryopreservation of transplant cells and tissue where it has been shown to increase viability [2] and decrease host immune response [9].

Building on extensive study in multiple biologic systems that describe its ability to inhibit lipid and protein misfolding [22], trehalose has become an attractive molecule for study in neurodegenerative disease characterized by protein misfolding and aggregate pathology. Such diseases include Alzheimer's and Parkinson's disease, and the less common triplet repeat diseases. Recent scientific publications describe trehalose benefit in model systems that recapitulate aggregate pathology that characterize Alzheimer's (AD), Huntington's (HD), and occulopharyngeal muscular dystrophy (OPMD).

EXPERIMENTAL

The mechanisms by which trehalose metabolism alters plant development are largely unknown. Trehalose itself could affect development by acting as signal molecule in carbohydrate metabolism. For example, trehalose induces enzymes of fructan synthesis in barley [27,17] and Sucrose synthase activity in soybean [20]. In general, sugars such as Sucrose and Glcose act as signals in the regulation of gene expression [15]. Whereas the expression of several source-specific genes is probably regulated by hexoses in a hexokinase-dependent signaling pathway [14; 13; 8], the regulation of the expression of some other genes appears to be directly mediated by Sucrose without prior cleavage to hexoses [30; 3; 21]. It is conceivable that trehalose, which is structurally similar to Sucrose, might act as an analog of Sucrose in sugar sensing. In addition to the trehalose that may be produced by the plants themselves, plants are also exposed to trehalose formed by microorganisms in mutualistic, as well as in pathogenic interactions. Trehalose formed by rhizobia during nodulation appears to have a strong effect on the carbohydrate contents in root nodules [19]. It is possible that trehalose-producing plant symbionts and/or pathogens can exploit the effects of trehalose to gain control over the sugar-sensing system of the plant. If this is the case, the trehalose-degrading enzyme trehalase, which is widespread among higher plants and is found in multiple tissues, may provide a safeguard against potentially deleterious effects of trehalose on carbohydrate allocation in plant-microbe interactions [18; 1].

Growth of Plants and analysis of Carbohydrates Using HPLC

The seeds were surface sterilized by immersion in sodium hypochloride (40%(v/v)) for 20 minutes, rinsed with distilled water, and transferred into plastic pots (8cm42 diameter) filled with perlite.The Seeds were planted in to sterile soil under conditions at 20°C with 4- 6 weeks light. Then extraction by coffee machine differents parts of plant: sach as flowers, roots, shoots and leaves. The insoluble pellets remaining from the carbohydrate extraction were resuspended in 0.2 mL of NaOH (0.5 M) and incubated at 60°C for 1 h. HCl (0.2 mL, 0.5 M) was subsequently added. After cooling down to room temperature, 0.6 mL of acetate/Na^+ buffer (0.2 M, pH 4.5) containing 1 unit of amyloglucosidase (special quality for starch determination, Boehringer Mannheim, Germany) was added and the samples was incubated overnight at 37°C. The reaction was stopped by boiling for 2 min. The samples were centrifuged (10 min at 10,000*g*); the supernatants were 10 times diluted, and were analyzed for Glc formation using HPLC.

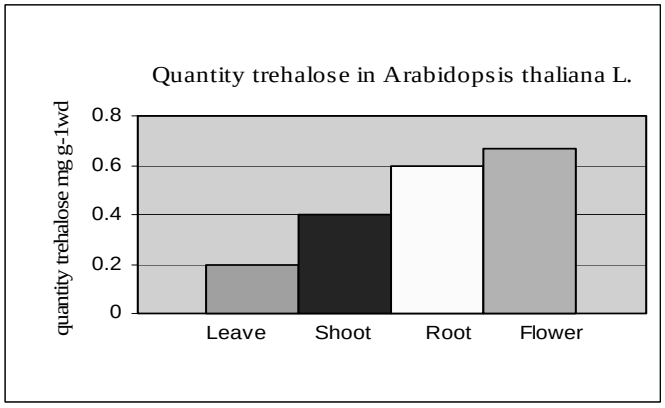
RESULTS AND DISCUSSION

Trehalose benefit was first shown in Huntington's model systems [25]. Huntington's is an autosomal dominant neurodegenerative disease, which presents with cognitive impairment, involuntary

choreiform movements, and psychiatric manifestations. Onset is generally in midlife, but can occur in childhood and old age. It inexorably progresses to disability and death over a 10-25 year period. Huntington's is characterized by an expanded CAG repeat within the first exon of the huntington gene. The mutant protein generated has an expanded polyglutamine (polyGN) tract. The pathologic hallmark of this and other polyGN diseases is the formation of aggregates, containing misfolded mutant protein in both cytoplasm and nucleus of affected cells. Tanaka, et al, demonstrated that trehalose inhibits polyglutamine-mediated protein aggregation of a model polyGN protein in vitro solution, and that it decreases aggregate formation and prolongs viability in a model cell culture. This same group went on to show that trehalose ameliorated motor symptoms, decreased aggregate number and size, and prolonged life by 20% in the R6/2 transgenic mouse model of Huntington's.

In *Arabidopsis*, inhibition of trehalase causes the accumulation of trehalose and a strong reduction in starch and Sucarus contents, suggesting a role for trehalose and trehalase in carbon allocation [16]. In addition, trehalose has been shown to inhibit *Arabidopsis* seedling root elongation and cause starch accumulation in shoots. Furthermore, trehalose increases AGPase (ADP-Glc pyrophosphorylase) activity and induces *APL3* gene expression [29, 10]. In soybean, trehalose also affects Suc synthase and invertase activities [20]. How trehalose affects plant gene expression, enzyme activities, photosynthetic activity, and carbon allocation is not clear, but trehalose-6-phosphate does not appear to have any effect on plant hexose phosphorylation [28]. However, transgenic tobacco plants expressing *Escherichia coli* homologs of TPS and trehalose-6-phosphate phosphatase show a positive correlation between trehalose-6-phosphate levels and photosynthetic activity, suggesting a regulatory role for trehalose-6-phosphate in plant carbohydrate metabolism [12].

In this study, *Arabidopsis thaliana L.* in the stress conditions, growthing was analysed different kinds of *Arabidopsis* for the presence of trehalose. Using as anion-exchange high performance liquid chromatography (HPLC) analysis, trehalose was highest in flower and root (figure below).



The distribution and activity of trehalase was measured in mature *Arabidopsis* grown. In these plants, a strong trehalase activity was found in mature flowers and roots, whereas leaves, stems, had significantly lower activities. Accordingly, its structure/activity benefits would be expected to persist for relatively extended times. If planned studies demonstrate brain or cerebral spinal fluid absorption, trehalose will open a new avenue of potential therapy for the prevention and treatment of multiple neurodegenerative diseases. This review summarized evidence for protective benefit in models of Huntingtin's disease, oculopharyngeal muscular dystrophy, and Alzheimer's. Although not studied, Parkinson's disease and amyotrophic lateral sclerosis display aggregate pathology that may be amenable to similar response.

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