

Why Can't Vertebrates Synthesize Trehalose?

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Abstract The non-reducing disaccharide trehalose is a singular molecule, which has been strictly conserved throughout evolution in prokaryotes (bacteria and archaea), lower eukaryotes, plants, and invertebrates, but is absent in vertebrates and—more specifically—in mammals. There are notable differences regarding the pivotal roles played by trehalose among distantly related organisms as well as in the specific metabolic pathways of trehalose biosynthesis and/or hydrolysis, and the regulatory mechanisms that control trehalose expression genes and enzymatic activities. The success of trehalose compared with that of other structurally related molecules is attributed to its exclusive set of physical properties, which account for its physiological roles and have also promoted important biotechnological applications. However, an intriguing question still remains: why are vertebrates in general, and mammals in particular, unable (or have lost the capacity) to synthesize trehalose? The search for annotated genomes of vertebrates reveals the absence of any functional trehalose synthase gene. Indeed, this is also true for the human genome, which contains, however, two genes encoding for isoforms of the hydrolytic activity (trehalase). Although we still lack a convincing answer, this striking difference might reflect the divergent evolutionary lineages followed by invertebrates and vertebrates. Alternatively, some clinical data point to trehalose as a toxic molecule when stored inside the human body.

Keywords Trehalose · Vertebrates · Invertebrates · Bacteria · Fungi · Plants

Introduction (overview)

Physical Properties of Trehalose

Trehalose is a ubiquitous disaccharide in the biosphere, where it fulfills essential physiological roles in a variety of prokaryotic and eukaryotic organisms (Table 1). The success of trehalose compared with other structurally similar sugars can be explained by its chemical structure as well as its remarkable and exclusive physical properties, which include high hydrophilicity and chemical stability (summarized in Table 1). The α,α -1,1-glycosidic linkage confers a non-reducing character to trehalose and makes it highly resistant to both acid hydrolysis and cleavage by glucosidases. Compared with other sugars, trehalose is more stable at a wide range of temperatures and pH (Richards et al. 2002). Together with its hydrophilicity and stability, trehalose displays a high degree of optical rotation and the melting point of the totally anhydrous trehalose is 203 °C. In water solution, trehalose can maintain a glass state that allows the formation of non-hygroscopic glass, which remains stable at high temperatures and when the glass becomes desiccated. Furthermore, the disaccharide also functions as a molecular chaperone in the efficient refolding of proteins partially denatured after severe environmental injury (Singer and Lindquist 1998; Crowe 2007). These features, together with the absence of internal hydrogen bonds, account for the principal role of trehalose as a stress metabolite, and confer on trehalose-producing organisms the potential to withstand drastic environmental perturbations, such as nutrient limitation, high temperatures, osmotic pressures, anhydrobiosis, or xenobiosis, for long periods (Table 1). Interestingly, trehalose accumulation is also induced upon cold exposure and is essential for the maintenance of cell viability at low temperatures

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Table 1 Summary of the main features displayed by the non-reducing disaccharide trehalose (O- α ,-D-glucopyranosyl-[1 \rightarrow 1]- α -D-glucopyranoside)

Physical properties	Physiological roles	Commercial use/applications
Non-reducing sugar	Energy and carbon reserve/source	Therapeutic compound
High hydrophilicity	Cell protectant against stress	Cryoprotectant
Internal chemical stability	Stabilizer of membranes and proteins	Prophylactic and stabilizer
Unable to form hygroscopic glasses	Component of the bacterial cell wall	Industrial fermentation
Absence of inner hydrogen bonds	Major compatible solute	Food sweetener
Resistant to acid hydrolysis	Sensing compound	Nutritional supplement
Elevated melting point	Virulence factor	Cosmetic additive

For other specific details, see the text and Ohtake and Wang (2011)

(Kandror et al. 2002). This ability explains its useful application as cryoprotectant in food, cosmetic, and pharmaceutical industries (Ohtake and Wang 2011; Table 1). The capacity to preserve cellular integrity against environmental changes arises from the stress-induced level of trehalose synthesis, regardless of the basal content of the disaccharide (Argüelles 2000; Richards et al. 2002; Crowe 2007; González-Párraga et al. 2011; Sánchez-Fresneda et al. 2014). Hence, trehalose is an ideal stabilizer to replace water, since it preserves both membranes and proteins in a physical state resembling fully hydrated conditions (Crowe 2007). Furthermore, due to its unique properties, trehalose has been intensively investigated for industrial and commercial purposes and is nowadays included in several commercially used therapeutic and cosmetic products (Ohtake and Wang 2011).

Physiological Roles of Trehalose in Prokaryotes and Eukaryotes

Different metabolic pathways, as well as several crucial (unrelated) functions, have been reported for trehalose (Fig. 1; Table 1), depending on the kind of biological system studied (Argüelles 2000; Elbein et al. 2003). As a general rule, it is a remarkable finding that prokaryotes show a more elaborated system for trehalose metabolism and the roles played by trehalose in prokaryotes are more varied than in eukaryotes. Thus, in bacteria, trehalose can be synthesized through at least three different pathways, termed the OtsAB, the TreS, and the TreYZ routes (a schematic representation is shown in Fig. 1; the interested reader should see the following reviews for more

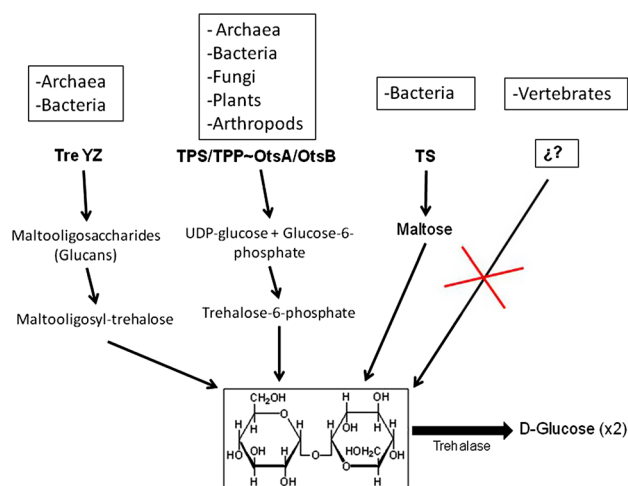


Fig. 1 Representative metabolic pathways involved in trehalose biosynthesis in the biosphere. The trehalose synthase (TS) pathway is reversible. To date, no route has been reported for trehalose synthesis in vertebrates. The hydrolysis of trehalose by trehalases releases two molecules of D-glucose. For other details, see the text

biochemical details: Elbein et al. 2003; Avonce et al. 2006; Tournu et al. 2013). Each specific pathway seems to be induced in response to different nutritional or environmental stimuli. However, it is quite common for all of them to coexist in a single bacterial species, even in several copies. It must, therefore, be assumed that there is tight regulation of these trehalose-synthesizing routes in bacteria to avoid redundant activity, although the pertinent molecular details remain largely unknown. In contrast, however, in lower eukaryotes, exemplified by yeasts and filamentous fungi, as well as in plants and invertebrates, the disaccharide is exclusively synthesized through the well-characterized trehalose-6P synthase/phosphatase (TPS/TPP) pathway (equivalent to the bacterial OtsAB one) (Tournu et al. 2013). The plants usually contain multiple TPS/TPP sequences, whose functionality is still not well understood (see below), whereas the presence in a yeast species of trehalose phosphorylase activity should be considered an exception (Argüelles 2000). In turn, a recently reported archaeal trehalose synthase complex matches the yeast mechanism (Zaparty et al. 2013).

The differences between prokaryotes and eukaryotes are even more conspicuous when the physiological roles of trehalose are examined. Thus, bacteria are capable of using trehalose as exogenous carbon source or as a structural component of the cell wall, where the “cord factor” composed of mycolic acids esterified with trehalose is the main inducer of pathogenicity in mycobacteria (Elbein et al. 2003). Furthermore, some species of bacteria and archaea can store the disaccharide as compatible solute, although other components (e.g., glycine betaines, K) are preferred for this role (Argüelles 2000). In the following

sections, the current status of trehalose metabolism in the above-mentioned organisms will be briefly surveyed.

Trehalose in Plants

Our knowledge of trehalose metabolism in plants has increased enormously in the last two decades. For a long time, it was believed that higher vascular plants lack the ability to synthesize trehalose at significant levels. Sucrose, the main transport sugar, seemed to fulfill a comparable role of storage and cell protection (Lunn et al. 2014; Delorge et al. 2014), while trehalose was regarded as an unusual sugar confined to the so-termed “resurrection” plants, which are subjected to prolonged periods of anhydrobiosis (Avonce et al. 2006). This picture has changed with the finding that trehalose metabolism is ubiquitous in plants, where it is synthesized by the well-established trehalose-6P-synthase/phosphatase pathway (TPS/TPP). In fact, plants possess an elaborated system that encompasses several trehalose biosynthetic genes, with distinct degrees of functionality (Eastmond and Graham 2003; Vandesteene et al. 2012). The intermediate trehalose-6-phosphate acts as key regulatory molecule in the control of growth and carbohydrate utilization, including starch degradation in leaves, and as developmental signal in embryo maturation, flowers, and young developing tissue (Schluepmann et al. 2003; Nunes et al. 2013; Mattos Martins et al. 2013; Delorge et al. 2014). The disruption of trehalose metabolism in plants causes pleiotropic phenotypes, involving alteration in stress tolerance, embryogenesis, leaf morphology, flowering, and inflorescence branching (Vandesteene et al. 2012; Lunn et al. 2014). More recent evidence points to a signaling role for trehalose in plant interactions with symbiotic microorganisms and herbivorous insects (Lunn et al. 2014). Despite some unwanted alterations, the heterologous expression of bacterial or yeast trehalose biosynthetic genes in some prototypic plants (*Arabidopsis*, rice or tobacco) increases stress tolerance (Lunn et al. 2014).

Trehalose in Invertebrates: (Insects, Nematodes, and Crustacean)

Because invertebrates are a very large, diverse, and heterogeneous group, very few representative examples have been chosen as research models. Below, we shall refer to the present knowledge regarding trehalose metabolism in those invertebrates. Trehalose has been isolated from the majority of insects so far examined (Wegener et al. 2010), where it is considered the major sugar in the circulatory fluid for insect growth and development and represents about 20 % of the total carbohydrate pool in specific stages

of development. The trehalose stored in the hemolymph is mobilized as a rapid fuel source for flight (Richards et al. 2002). Furthermore, most insect species have evolved a period of growth arrest called diapause, which ensures their survival through seasonal periods of environmental adversity (Xu et al. 2009). This diapause stage resembles the resting or dormant states observed in bacteria and fungi. In fact, the activity of trehalose-6P synthase and the trehalose content are much higher in diapause-destined individuals than in non-diapause individuals (Xu et al. 2009). In *Drosophila melanogaster*, the synthesis of huge amounts of endogenous trehalose triggered by expression of the trehalose synthase (*tps1*) gene increased tolerance to hypoxic and anoxic injuries (Chen and Hadadd 2004).

In the prototypical nematode *Caenorhabditis elegans*, trehalose is present throughout the life cycle in significant amounts (up to 2.5 % dry weight), the greatest concentrations being found in eggs and dauer larvae, two stages that display high resistance to environmental stress. The metabolic pathways in *C. elegans* seems rather complex, two putative genes encode trehalose-6P biosynthesis (*tps*) and five putative trehalase (*tre*) genes code for the enzymes responsible for disaccharide hydrolysis (Pellereone et al. 2003). Of note is the fact that studies of organismal aging in *C. elegans* revealed the ability of trehalose to extend the longevity of this nematode without any relevant side effects (Honda et al. 2010).

Detailed work on trehalose metabolism has also been carried out in the crustacean *Artemia franciscana*. The encysted embryos (cysts) store large quantities of the sugar, which acts as protectant against heat shock, desiccation, and anoxia, although it did not provide specific protection against oxidative damage. When dormancy is broken, a fraction of trehalose is quickly mobilized as source of energy, whereas the remainder serves as a substrate for glycogen and glycerol synthesis (Collins and Clegg 2004). On the other hand, the phylum apicomplexans, a group of protists evolutionarily close to dinoflagellates and ciliates, is able to synthesize trehalose through the same biosynthetic pathway as characterized in fungi and plants (Yu et al. 2010).

Trehalose and Trehalose-Synthesizing Genes are Absent in Vertebrates

The evolutionary trend that conserved trehalose as key biological molecule is abruptly abrogated with the invertebrates. Interestingly, emergent vertebrates do not appear to have needed the disaccharide for their successful development in the biosphere and, to date, not one vertebrate species has been reported with the ability to store trehalose. Even more perplexing, not one gene involved in trehalose biosynthesis has been annotated in the customary

genome data bases for vertebrates (in theory, other alternative pathways could be operative besides the classical trehalose synthase and trehalose phosphatase itineraries). An extensive search in the genome of some representative vertebrates (human, rat, mouse, or rabbit) was carried out using as template, the published sequence of trehalose synthase genes (*TPS1* and *TPS2/TPP*) chosen from several model organisms, namely yeasts, *Arabidopsis*, and *C. elegans*. A more exhaustive analysis of synteny is necessary to determine the differences in genomic organization between vertebrates and non-vertebrates organisms, like the detailed studies on genetic collinearity performed in *Arabidopsis* (Vandesteene et al. 2012). Therefore, apart from plants, the capacity to produce and accumulate trehalose has been exquisitely conserved throughout the progression from prokaryotes to lower eukaryotes until all known invertebrates (within the animal kingdom). Later, evolution seems to have excluded the ability in vertebrates to synthesize trehalose, although the capacity to hydrolyze the disaccharide has been maintained.

Why is trehalose absent in vertebrates and, more specifically, in mammals? The possible reasons for the absence of trehalose are intriguing, since apart from being a stable molecule with a role as protectant compound, this non-reducing disaccharide (two glucose units linked in an α,α -1,1 configuration) might provide on-demand a soluble source of glucose, the main blood sugar of vertebrates, whose concentration must be maintained within strict limits. This question immediately leads to another regarding whether vertebrates have lost the capacity to synthesize trehalose or whether actually they never acquired it. The definitive answer is not obvious, but the second option seems to be more likely based on the available evidence, although there is still room for reasonable speculation and objections. In evolutionary terms, this outstanding difference concerning trehalose would not be surprising, because invertebrates and vertebrates have a markedly divergent lineage and have followed distinct lines since the early steps of evolution. Whereas, most invertebrates come from protostomes, vertebrates (together with some invertebrates like the Echinodermata) are derived from deuterostomes. Neither in Echinodermata nor in *Branchiostoma* (primitive chordates) has any trehalose-synthesizing gene been reported in the customary genome data bases, despite some preliminary description in these organisms of enzymatic activities that hydrolyze the disaccharide (Scheibling 1980). Thus, trehalose might be a conspicuous marker that reinforces this divergent origin of embryonic development.

An extensive and rigorous phylogenetic analysis of trehalose biosynthesis (Avonce et al. 2006) revealed that in most eukaryotic organisms, trehalose-6P-synthase (TPS) and trehalose-6P-phosphatase (TPP) proteins are fused in a

single domain, supporting the idea that both descend from a common ancestor. However, to date, no functional enzyme with simultaneous Tps and Tpp activities has been reported in eukaryotic organisms, although a bifunctional Tps–Tpp protein has been reported in the bacterium *Cytophaga hutchinsonii* (Avonce et al. 2010). This prokaryotic system might be the earliest precursor of the eukaryotic trehalose biosynthesis genes (Avonce et al. 2010). Of note is the fact that the above exhaustive phylogenetic studies do not explain why the evolutionary path is unexpectedly interrupted in vertebrates.

The picture is even more complicated because, contrary to the lack of trehalose biosynthetic genes, two trehalose-hydrolyzing activities are present in vertebrates. These activities in humans display precise locations, acting as intrinsic glycoproteins of the microvillus intestinal mucosa and renal brush-border membranes linked by a GPI-anchor, as shown by their selective solubilization by phospholipase C (Ishihara et al. 1997). This dual enzymatic activity points to a strict control and rapid degradation of the trehalose ingested in the diet, preventing it from being accumulated even in transitory or low levels. Intestinal trehalase is with all probability the sole enzyme responsible for the hydrolysis of ingested trehalose (mushrooms and honey among other foods, are rich in the non-reducing disaccharide). Alterations of this catalytic activity cause important digestive disorders (see below). Notably, intestinal trehalase is never released into the bloodstream and is tightly attached to the external surface of the microvilli of enterocytes, resisting all proteolytic treatments (Richards et al. 2002).

In turn, the physiological role of trehalase in kidney remains more elusive, since human blood does not contain trehalose, which is the major sugar in the hemolymph of insects, where it is indispensable for flying and a variety of functions that require rapid or instantaneous energy availability. The pioneering work of Sacktor (1968) and Van Handel (1969) studied a hypothetical role for renal trehalase in the reabsorption of glucose via trehalose synthesis and subsequent transport of hexoses in the kidney. This role, however, better fits with a trehalose synthase rather than with a hydrolytic activity.

Is Trehalose a Toxic Molecule for Vertebrates?

As an alternative, compatible, and feasible explanation, some evidence from clinical assays suggests that the accumulation of trehalose or its derivative trehalose-6P may induce toxicity (Buts et al. 2008). Hence, trehalose ingested in the diet cannot directly be assimilated as such into the blood stream. Rather, it is hydrolyzed by intestinal trehalase and the two D-glucose moieties are subsequently absorbed and metabolized by the same mechanism evolved

by the body to take up other assimilable disaccharides. Thus, trehalose as a dietary ingredient should be estimated as safe for nutrition, although several studies dealing with diets supplemented with a high trehalose content are far from conclusive (Richards et al. 2002). Furthermore, elevated levels of intestinal trehalase activity are recorded during gestation, birth, and after weaning; however, the metabolic capacity to digest dietary trehalose remains roughly equivalent throughout life in healthy children and adults (Richards et al. 2002).

Nevertheless, a major role for intestinal trehalase is undisputed, since patients suffering trehalose intolerance caused by deficiency of this activity suffer abdominal symptoms and diarrhea. The malabsorption or intolerance to trehalose is transient and successfully remedied upon addition of a probiotic (*Saccharomyces boulardii*), which releases trehalase in the intestinal lumen (Buts et al. 2008).

Another argument that supports the toxicity of trehalose is related with its particular physical properties (see Table 1). The molecule is virtually inert, with low reactivity, highly resistant to acid hydrolysis and melts at 203 °C, suggesting the accumulation of trehalose in large concentrations would be dangerous. In fact, in yeasts that have overcome heat shock, trehalose acts as chaperone preventing the degradation of partially denatured proteins, but it must be quickly degraded later in order to ensure proper heat shock recovery (Singer and Lindquist 1998). Finally, the absence of trehalose in humans does not preclude its application to improve the in vitro tolerance of human cells to desiccation, and to enhance platelet preservation or the longer viability of embryos and other tissues for cryopreservation (Crowe 2007).

Implications and Conclusions

Leaving plants aside, and although the experimental data are still clearly insufficient, it is tempting to envisage a theoretical proposal concerning an important role of trehalose in unicellular organisms in ensuring proper cell growth and viability under challenging conditions, while being dispensable or even toxic in multicellular animals. Thus, unicellular organisms have evolved efficient mechanisms for adaptation to adverse nutritional or environmental circumstances (e.g., dehydration, high temperature, or osmotic pressures, the presence of toxic compounds or oxidants). Intracellular storage of large amounts of trehalose might be one of the well-established mechanisms that explain the physiological tolerance and preservation of cell integrity against such extreme environmental injuries. Thus, trehalose is synthesized as a compatible solute by a number of bacteria and archaea, as well as by single structures (spores, germlings, and myxoamoebae) and the resting states of some filamentous multicellular fungi.

Supporting this proposal, the introduction of genes coding for trehalose biosynthetic enzymes into plant cells by genetic engineering increased their resistance to drought, desiccation, and prolonged their half life (Eastmond and Graham 2003; Vandesteene et al. 2012; Mattos Martins et al. 2013; Lunn et al. 2014). Previous efforts involving molecular engineering of mammalian cells have demonstrated the only partial success of trehalose for preserving fibroblasts in dry states (Guo et al. 2000), although it performed better in prolonging freeze-dried platelets (Crowe 2007).

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