Trehalose – properties, biosynthesis and applications

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Introduction

A few decades ago it was thought that carbohydrates are just the source of energy. However, over the years it was revealed that many of them fulfill specialized biological functions. Trehalose, which was described for the first time in the 30s of the nineteenth century, turned out to be an important sugar with the ability to protect proteins during environmental stresses [1]. This discovery resulted in an increased interest in trehalose and current number of research papers mentioning this disaccharide exceeds 10 000 documents (Scopus). Although bioprotective properties of trehalose have been well known for many years, it was not obtained on a large scale for a long time due to the high costs of production. Its price has decreased enough after the development of its enzymatic manufacturing from poly- and oligosaccharides making possible to use it in various industries. In the recent years, numerous reviews on trehalose have been published describing its unique properties and applications [2 – 6], mechanisms of biosynthesis [7 – 8] as well as its production by the biotechnological methods [3, 9 – 10].

Chemical structure

Naturally occurring trehalose is composed of two glucose molecules linked by α,α-1,1'-*O*-glycosidic bond. It has the lowest conformation energy among the three possible isomers: α,α-, α,β-, β,β- (Fig. 1). In addition, due to generally low energy of glycosidic bond (lower than -4.2 kJ•mol^{-1}) it is thermodynamically and kinetically the most stable disaccharide found in nature. It is also important, that D-glucopyranosyl units are linked involving anomeric carbons and consequently trehalose do not possess reducing properties [3].

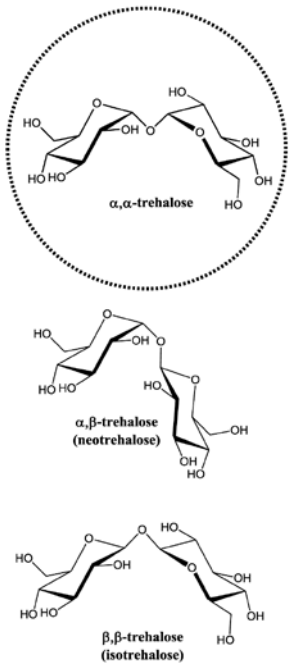

Fig. 1. Chemical structure of trehalose isomers

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Natural occurrence and properties

Trehalose is widely spread in nature. It is present in bacteria, fungi, plants and in many invertebrates such as nematodes, crustaceans and insects [2, 3]. Although its biosynthesis in mammals are not known, significant amounts of trehalase, an enzyme cleaving trehalose into two glucose molecules, was found in human small intestine [5]. Trehalose is mainly known from its ability to protect proteins and other biomolecules under stress conditions: in very high and very low temperatures, during dehydratation or under osmotic stress [2, 11]. It was reported that popular food yeast *Saccharomyces cerevisiae* have synthesized greater amounts of trehalose in the response to heat shock. When temperature decreased its concentration declined to the initial level. Great amounts of trehalose (approx. 15 % dry weight) are also accumulated by organism capable of anhydrobiosis [12]. Typical examples are resurrection plants, with the most well-known rose of Jericho, which could survive many years in the state of complete dehydratation and then recover their normal physiological activity.

Three theories are proposed to explain why trehalose enables proteins to withstand extreme environmental conditions. They are not mutually exclusive and currently it is claimed that protective mechanism depends on the abiotic stress factor and the way it works [2 – 3, 13 – 14]. The water replacement theory suggests that trehalose may protect proteins during dehydratation or freezing by replacing water molecules which normally forms hydratation layer around biological structures. It is a result of great flexibility of glycosidic bond that allows trehalose to interact with the irregular polar groups of biomacromolecules. According to the water entrapment theory, trehalose rather than biding directly to biomolecules, entraps water close to their structure maintaining their native hydratadion. The vitrification theory proposes, that it forms a non-hygroscopic glassy state with high temperature resistance and high stability also when it is essentially completely desiccated. This glassy matrix may hold proteins and protects their secondary, tertiary and quaternary structure from unfolding and thus loss of bioactivity.

Chemical synthesis, biosynthesis and manufacturing of trehalose

Chemical synthesis of α,α-trehalose by heating of 2,3,4,5-tetra-O-acetyl-D-glucose with 3,4,6-tri-O-acetyl-1,2-anhydro-D-glucose in benzene was reported by Lemieux in 1954 [15]. There are known some other methods of α,α-trehalose synthesis, but due to low yield and lack of stereoselectivity, only biotechnological processes are utilized in large-scale prepatations.

Until 1990s trehalose has been produced in relatively small amounts by extraction from yeast and vegetable sources. Multistep procedures and low yield made the process very costly and consequently trehalose was only available for research and biomedical applications in small amounts. α,α-Trehalose is the only anomer of trehalose that can be biosynthesized in many different types of organisms. Literature data show at least five different pathways for the biosynthesis of trehalose (Fig. 2) [8]. Pathway A, the most widely distributed among them (Fig. 2. A), was discovered about 55 years ago, and it has been reported in eubacteria, archaea, fungi,

insects and plants [16]. It involves two enzymatic steps catalyzed by trehalose-6-phosphate synthase (TPS) and trehalose-phosphatase (TPP). TPS catalyses the reaction of UDP-glucose with glucose-6-phosphate to produce trehalose-6-phosphate, which is hydrolyzed by TPP to trehalose. The second biosynthetic pathway (Fig. 2. B) was reported in bacteria capable of utilization of starch, glycogen or maltodextrins as a starting material for trehalose synthesis. Trehalose was synthesized by sequential action of two enzymes: maltooligosyltrehalose synthase (TreY) and maltooligosyltrehalose trehalohydrolase (TreZ) [7]. TreY acts on the last α-1,4-glucosidic bond at the reduced end of maltodextrins, which is converted into an α,α-1,1'-glycosidic bond, forming maltooligosyltrehalose. Next, TreZ catalyses the hydrolysis of the second α-1,4-glucosidic bond of maltooligosyltrehalose and trehalose is released. In some bacteria, e. g. *Pimelobacter sp.*, the enzyme trehalose synthase (TreS) catalyzing an intermolecular rearrangement of maltose to trehalose was found (Fig. 2. C) [5, 7]. TreS isomerizes the α-1,4-glycosidic bond of maltose to a α,α-1,1'-glycosidic bond, forming trehalose. In the fourth pathway, trehalose phosphorylase (TreP), present in some fungi, catalyzes the reversible hydrolysis of trehalose (Fig. 2. D) [9]. TreP catalyzes the hydrolytic release of trehalose from trehalose-6-phosphate, previously formed from glucose and glucose 1-phosphate. The last biosynthetic pathway, was found recently in the hyperthermophilic archaeon *Thermococcus litoralis*, and involves the trehalose glycosyltransferring synthase (TreT), which catalyses the reversible formation of trehalose from ADP-glucose and glucose (Fig. 2. E) [17 – 18].

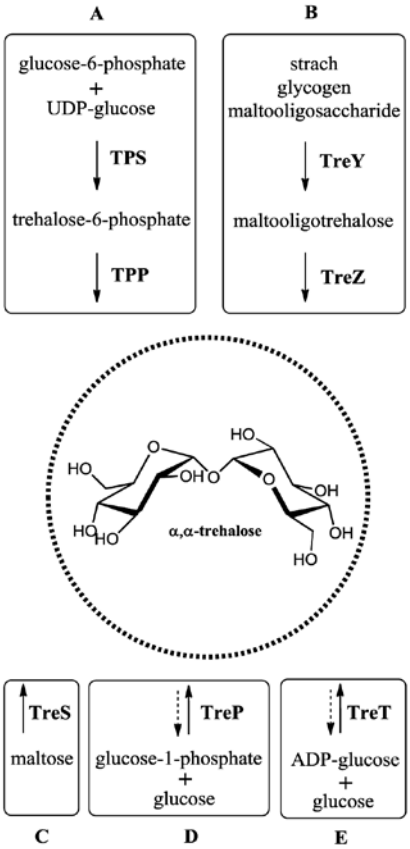

Fig. 2. Biosynthesis of trehalose, mechanisms of bioconversion of different substrates Abbreviations: TPP – trehalose 6-phosphate phosphatase, TPS – trehalose 6-phosphate synthase, TreP – trehalose phosphorylase, TreS – trehalose synthase, TreT – trehalose glycosyltransferring synthase, TreY – maltooligosyl-trehalose synthase, TreZ – maltooligosyl-trehalose trehalohydrolase

In principle, three biosynthetic pathways: A, B and C (Fig. 2) could be used for biotechnological manufacture of trehalose. The development of biotechnological method depends on availability of raw material and source of enzymes. Several reports on large-scale industrial production of trehalose comes from Japanese concern Hayashibara Co., Ltd. in Okayama. In 1995 they started industrial process, based on biosynthetic pathway B (Fig. 2), which utilizes starch from maize or tapioca and enzymes from *Arthrobacter ramosus* [19]. By 2005, the production unit yielded about 30 000 ton 98% pure trehalose dihydrate yearly [1]. After several improvments, modern biotechnologies have reduced the production costs to less than 3 $ per kg [3]. For comparison, cost of 1 kg trehalose in 1990 was almost 700 $ [20].

Applications

After the development of large scale biosynthesis, trehalose has found numerous applications in various areas, mainly in the food, cosmetic and pharmaceutical industry as well in medicine. It is also observed that new reports on its potential uses continues to grow [3, 5].

Trehalose is Generally Regarded As Safe (GRAS) by the U.S. Food and Drug Administration [14]. Moreover, it could be accumulated in body fluids in large quantities without exhibiting toxicity. It is consumed as a part of the human diet, as it is present for example in bread, honey, mushrooms, wine or beer. Trehalose, unlike sucrose is resistant to non-enzymatic browning which is mainly the result of Maillard reaction and carmelization [21]. Since it is a non-reducing sugar it do not undergo Maillard reaction with compounds containing amino groups such as amines, amino acids, peptides or proteins. Resistance to carmelization is in turn the result of low energy of glycosidic bond, what determines its high stability and low susceptibility to hydrolysis into glucose. This makes trehalose an attractive ingredient for the food industry. It is used as an sweetner and bulking agent. It has the ability to mask unpleasant malodors and off-tastes as well to preserve starch, lipids, and proteins against degradation by oxidation, heating or cooling. It is also added to dried or processed fruits and vegetables in order to preserve their aromas and organoleptic properties [3, 5, 21].

In medicine trehalose is a key ingredient of the solutions used for cryopreservation of stem cells as well for effective preservation of organs and tissues for transplantation. Moreover, it is an additive, which prolongs shelf-life of vaccines and antibodies and in case of numerous thermolabile enzymes such as DNA polymerase or restriction enzymes allows to store them at ambient temperatures [3, 21]. Studies have shown, that daily ingestion of trehalose-containing food has a beneficial effect for bone metabolism and could prevent osteoporosis [5].

In pharmaceutical products trehalose is employed as an excipient in protain-based drugs including Advate®, Avastin®, Lucentis® and Herceptin® [22]. It is also an ingredient of drops THEALOZ® used for the treatment of dry eye syndrome, since it exhibits ability to protect corneal epithelial cells from death by desiccation and suppress tissue denaturalization [5].

In the cosmetic industry trehalose has found an application as a moisturizing ingredient, due to its protective properties towards liposomes contained in cosmetic products as well lipids and proteins presented in the skin [3, 21]. It is proposed to use trehalose in deodorants for elderly people [5]. Studies have shown, that its 2% solution could reduce unpleasant odor emitting from human skin by up to 70%. It is due to the suppressive effect on degradation of unsaturated fatty acids to certain aldehydes with characteristic odor.

Polymers bearing trehalose

In many neurodegenerative disorders like Alzhaimer's, Parkinson's or Huntington's disease, it was found that abnormal aggregation and deposition of specific proteins occurs. Numerous studies have proven that trehalose limits these process and inhibits formation of neurotoxic amyloids [23 – 25]. Moreover, reports

presented by Miura et. al. [26 – 27] have shown, that these properties are amplified when trehalose is incorporated in polymer chain as a pendant group (Fig. 3. A). Similar results were obtained by Maynard et. al. [14, 22] whose studied stabilization effect of trehalose and its glycopolymers on enzymatic activity of selected proteins exposed to environmental stresses.

Therapeutic proteins and polypeptides are commonly used to treat broad spectrum of disorders like arthritis, anemia, diabetes or cancer [28]. Unfortunately, their fragile three-dimensional structure makes them very susceptible to proteolytic and chemical degradation as well as physical unfolding and aggregation in body fluids, what leads to the fast loss of their bioactivity. Therefore, frequent injections or infusions limiting patient's comfort are necessary to obtain a therapeutic effect [29]. Moreover it is well recognized that protein inactivation could result in an enhanced immune response, which could be also directed at endogenous proteins [30]. One of the strategy used to increase in vivo stability of therapeutic proteins is to employ hydrogel carriers within which they are entrapped and from which they are released in controlled manner [28]. Recently, hydrogels cross-linked by trehalose diacetals have been proposed as novel protein carriers (Fig 3. B) [31]. Due to the sensitivity of acetal bond to acid-catalyzed hydrolysis, obtained materials degraded in acidic medium, what was accompanied by trehalose release. Taking into account its bioprotective properties, it is expected that it would be able to form protective microenvironment preventing inactivation of simultaneously released proteins.

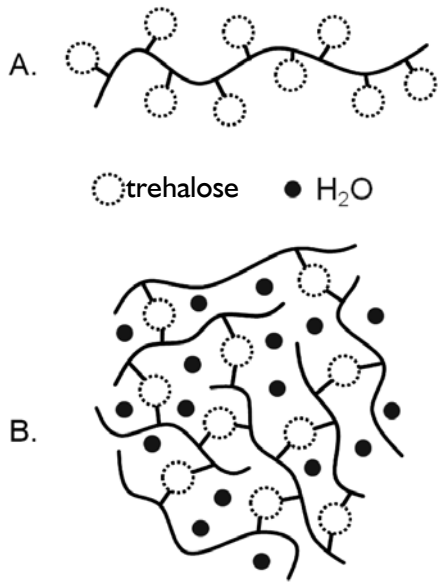

Fig. 3. A. Trehalose glycopolymer B. Hydrogel cross-linked by trehalose derivatives

Summary

Due to its unique bioprotective properties, trehalose becomes more and more frequently used in many applications, e. g.: stabilization of proteins, tissue protection, as a moisturizer in cosmetic preparations and in food chemistry. The utilization of trehalose in so many fields is possible by advances in the discovery of suitable biocatalyst to be used in the large-scale production. After several improvements, modern biotechnologies based on enzymatic transformation of starch have reduced the production costs of trehalose to about 3 $ per kg.

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Synthos SA i NCBR wspólnie na rzecz innowacji w polskim sektorze chemicznym

Narodowe Centrum Badań i Rozwoju i Synthos SA podpisały porozumienie, na mocy którego uruchomią oparte na formule *open innovation* przedsięwzięcie dedykowane branży chemicznej. Na wsparcie prac badawczo–rozwojowych przeznaczą 200 mln PLN. Realizacja wspólnego przedsięwzięcia przez NCBR oraz Synthos SA ma na celu opracowanie i wdrożenie nowej generacji produktów chemicznych, które przyczynią się do wzrostu innowacyjności polskiej branży chemicznej. Dzięki współpracy NCBR z Synthos SA wsparcie otrzymają najlepsze projekty B+R. *(kk)*

(http://synthosgroup.com, 21.07.2015)

Polska – krajem członkowskim ESO

Polska może już decydować o największej infrastrukturze i projektach astronomicznych na świecie. Zakończył się formalny proces ratyfikacji członkostwa Polski w Europejskim Obserwatorium Astronomicznym na Półkuli Południowej. To oznacza, że jesteśmy już krajem należącym do tej organizacji. Polscy naukowcy będą korzystać z najnowocześniejszych na świecie instrumentów badawczych i aparatury do naziemnej obserwacji Kosmosu. Do ESO należy 15 państw, obok Polski, między innymi Francja, Hiszpania, Niemcy, Wielka Brytania i Włochy, a spoza Europy także Brazylia. Dzięki współpracy w ramach organizacji, naukowcy prowadzą obserwacje z wykorzystaniem potężnych teleskopów, których budowa przekraczałaby możliwości jednego kraju. *(kk)*

(http://www.nauka.gov.pl/, 5.08.2015)

Superkomputer ze Świerku

Klaster komputerowy zbudowany w Narodowym Centrum Badań Jądrowych został oficjalnie zaliczony do grona najszybszych superkomputerów na świecie. Stworzona w ramach projektu Centrum Informatyczne Świerk instalacja znalazła się na 155. miejscu prestiżowej listy TOP500. Stworzony w ramach projektu Centrum Informatyczne Świerk superkomputer, to obecnie jedna z czterech najszybszych instalacji obliczeniowych w Polsce. Tworzy go m.in. 1916 procesorów (19 880 fizycznych rdzeni obliczeniowych), wspieranych przez 119,75 TB pamięci RAM oraz ponad 2,9 PB przestrzeni dyskowej. Równolegle z budową infrastruktury obliczeniowej stworzono interdyscyplinarny zespół naukowców oraz ekspertów. Głównym celem projektu CIŚ jest wsparcie rozwoju polskiej energetyki – zarówno jądrowej, jak i konwencjonalnej. Umiejętności specjalistów oraz możliwości superkomputera wykorzystywane są na przykład przy projektowaniu urządzeń energetycznych, optymalizowaniu procesu dystrybucji energii, analizowaniu bezpieczeństwa reaktorów jądrowych, a także monitorowaniu i symulowaniu zagrożeń radiacyjnych oraz wspieraniu zarządzania kryzysowego. W projekcie prowadzone są też badania naukowe i rozwojowych dotyczących m.in. eksploatacji reaktorów jądrowych, analiz zagrożeń chemicznych oraz modelowania układów złożonych. *(kk)*

(http://www.ncbj.gov.pl/, 20.07.2015)

ORLEN i PGNiG wspólnie poszukają gazu i ropy na Podkarpaciu

ORLEN Upstream przystąpił do Umowy o Wspólnych Operacjach z Polskim Górnictwem Naftowym i Gazownictwem SA (PGNiG). Porozumienie zakłada wspólną realizację prac analitycznych i badawczych na obszarze ośmiu bloków koncesyjnych w obrębie województwa podkarpackiego. Celem wspólnych operacji jest poszukiwanie, rozpoznawanie oraz wydobycie ropy naftowej i gazu ziemnego w rejonie Karpat. Na mocy zawartego porozumienia, ORLEN Upstream obejmie 49% udziałów w ośmiu blokach koncesyjnych o wspólnej nazwie projektowej „Bieszczady". Umowa zakłada realizację uzgodnionego przez partnerów programu prac. *(kk)*

(http://www.orlen.pl/, 20.07.2015)

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