Thermotolerance in Saccharomyces cerevisiae: the Yin and Yang of trehalose

Mike A. Singer and Susan Lindquist

Trehalose, a sugar produced by a wide variety of organisms, has long been known for its role in protecting certain organisms from desiccation. Recent work in yeast indicates that trehalose also promotes survival under conditions of extreme heat, by enabling proteins to retain their native conformation at elevated temperatures and suppressing the aggregation of denatured proteins. The latter property, however, seems to impair the recovery of cells from heat shock if they fail to degrade trehalose after the stress has passed. These multiple effects of trehalose on protein stability and folding suggest a host of promising applications.

The disaccharide trehalose was first discovered incidentally, from what might appear to be an unlikely source: ergot, a fungal crop blight (Fig. 1a). Ergot, which affects rye and other cereals, has plagued civilization throughout recorded history. The Bible, as well as classical Greek literature, makes reference to this ascomycete, whose toxins can cause insanity and death. Ergot drew the attention of H. A. L. Wiggers who, in 1832, identified among its constituents a previously unknown sugar. A quarter–century later, M. Berthelot found the same sugar in cocoons of the beetle Larinus (Fig. 1b) that were used for medicinal purposes in what was then Persia and the Ottoman Empire. These cocoons were called ‘trehala’, inspiring Berthelot to name the sugar ‘trehalose’ (Box 1).

Trehalose has since been found in a great variety of species, most notably the anhydrobionts. These unusual organisms, which include ‘resurrection plants’ (Fig. 2), certain brine shrimp, nematodes and baker’s yeast (Saccharomyces cerevisiae), are able to withstand almost complete desiccation. They endure long periods in seemingly suspended animation, returning from dormancy to full activity once water again becomes available. Both how trehalose facilitates their survival and how it may be used for the storage of food or labile biological materials have been previously considered.

Recent work in S. cerevisiae now indicates that trehalose is also crucial for survival from a very different kind of stress: high temperatures. Heat damages cells in a variety of ways, perhaps most critically by disrupting the integrity of membranes and by causing proteins to denature and aggregate. Although trehalose has been shown in other contexts to stabilize membranes and native proteins, the maintenance of denatured proteins has long been thought the province of heat-shock proteins (Hsp). Some Hsp function as molecular chaperones, preventing protein aggregation and restoring denatured proteins to their native state. For example, Hsp70 binds extended hydrophobic amino acid sequences that are normally sequestered in the protein core; such regions, which are prone to aggregate, become exposed when proteins denature. Hsp104p, the yeast protein principally responsible for severe-stress tolerance, is unusual in that it mediates the resolubilization of polypeptides that have already begun to aggregate. Other Hsps promote recovery from heat shock by facilitating the degradation of irretrievable damaged proteins. Here, we discuss recent efforts in S. cerevisiae and in vitro that have advanced our understanding of trehalose synthesis and degradation, and helped to illuminate the mechanisms by which trehalose promotes survival during heat shock. These findings are applicable to other stresses and other organisms, and suggest a wide variety of promising applications.

The synthesis of trehalose

Trehalose (α-D-glucopyranosyl-α-D-glucopyranose) is a nonreducing disaccharide composed of two molecules of glucose linked at their 1–carbons. Although barely detectable in log-phase yeast growing on glucose, trehalose accumulates to remarkably high levels – up to 20% of the dry weight of the cell – in stationary-phase cells and spores, as well as in exponential-phase cells exposed to high temperatures. Cells growing on nonfermentable carbon sources have high levels of trehalose in both log and stationary phase. For a comprehensive treatment of the relationship between trehalose and cell physiology in S. cerevisiae, see Ref. 11.

The enzymes catalysing the synthesis of trehalose (Table 1) are localized to the yeast cytosol, where production of the sugar occurs in two steps (Fig. 3): first the glucose moiety from uridine-diphosphate–glucose is joined to glucose-6-phosphate, forming trehalose-6-phosphate; the phosphate group is then removed to yield trehalose and recycle free inorganic phosphate.

The enzymatic complex that carries out this process consists of at least three distinct, copurifying components – Tps1p, Tps2p and Tsl1p (Refs 14–18). A fourth protein, Tps3p (identified by sequence homology with Tsl1p), has also been found to associate with Tps1p and...
Tps2p (S. Hohmann, pers. commun.). Tps1p catalyses the first step in trehalose synthesis; cells lacking this gene produce neither trehalose nor trehalose-6-phosphate. Phosphatase activity resides in Tps2p; tps2 mutants accumulate large quantities of trehalose-6-phosphate under circumstances in which trehalose would normally be found. The functions of Tsl1p and Tps3p are less clear, but these proteins seem to contribute to regulation: either gene can be deleted without affecting the heat-induced accumulation of trehalose by log-phase cells, but accumulation is severely impaired if both Tsl1p and Tps3p are absent. How these subunits combine to form the functional enzyme remains unknown. Estimates based on gel filtration place the size of the complex at about 630–800 kDa, signifying that one or more constituent is present in more than one copy. Two-hybrid analysis indicates that Tps1p and Tps2p, the catalytic components of the enzyme, interact both with themselves and with one another; the putative regulators Tsl1p and Tps3p do not interact with each other, but each binds Tps1p and Tps2p. Tps3p also self-associates. Association of Tps3p and Tsl1p with the trehalose synthase complex may be coordinated with certain growth conditions: expression of TPS3 is constant in log and stationary phase, whereas that of TSL1 increases as cells enter stationary phase. The factors regulating trehalose levels under different conditions are poorly understood. Tps1p, Tps2p and Tps3p are present constitutively, and the expression of TPS1 and TPS2 is further induced by heat. The increased production of trehalose during heat shock appears to have several causes. The activity of the enzyme complex increases with heat, but it is not certain whether the activity is influenced by phosphorylation. Trehalose levels are known to increase, at least in part, through the synthesis of additional Tps1p and Tps2p, as well as through a rise in the intracellular levels of the substrates UDP–glucose and glucose-6-phosphate.

TPS1 and growth on glucose

An unexpected, and as-yet unexplained, phenotype of tps1 mutants is their inability to grow on readily fermentable carbon sources, such as glucose. When placed in glucose-containing media, the cells become flooded with the sugar, which is readily phosphorylated by hexokinases to yield vast quantities of glucose-6-phosphate. Levels of ATP and free inorganic phosphate consequently plunge, presumably bringing critical cellular functions to a standstill. Three explanations have been proposed to account for this phenotype (for a detailed review, see Ref. 28), and are currently being investigated. The first is based on the in vitro observation that trehalose-6-phosphate inhibits the activity of hexokinases; as this regulation is lacking in tps1 mutants, uncontrolled phosphorylation would result, with its ancillary toxic effects. A second model suggests that trehalose synthesis serves as a buffer system for glycolysis. Conversion of excess glucose-6-phosphate into trehalose would recycle free inorganic phosphate for later use in glycolysis, where it is needed to generate glyceraldehyde-3-phosphate from 1,3-bisphosphoglycerate. Finally, Tps1p may, in addition to its role in trehalose synthesis, have a second function directly influencing the transport or phosphorylation of sugars.

Trehalose catabolism

Trehalose can be degraded by various trehalases (Table 1), which hydrolyse the disaccharide to yield...
two molecules of glucose. Although trehalose has been detected exclusively in the nuclear–cytosolic compartment, trehalase activity is present both in this region and in the vacuole. The vacuolar enzyme is constitutively active, with its pH optimum at 4.5 ("acid trehalase"). The activity of the cytosolic protein is maximal at pH 7 ("neutral trehalase") and is regulated by phosphorylation.

The **ATH1** gene, encoding acid trehalase, is expressed in stationary phase but not during exponential growth. The enzyme’s activity level does not increase significantly with elevated temperature. During stationary phase, ath1 mutants have been reported to show increased trehalose levels and enhanced survival of dehydration, freezing or exposure to ethanol. Of course, as Ath1p is vacuolar and trehalose is cytosolic, the mechanism by which Ath1p influences the trehalose content is not readily apparent. Unlike wild-type cells, ath1 mutants are unable to utilize exogenous trehalose as a carbon source, which is consistent with extracellular trehalose reaching the vacuoles by membrane transporters, endocytosis or autophagocytosis.

During recovery from heat shock or exit from stationary phase, primary responsibility for trehalose hydrolysis rests with the cytosolic trehalase Nth1p. Disruption of the gene causes cells growing at elevated temperatures to accumulate higher levels of trehalose, which then persist upon return to a normal temperature. NTH2, a gene 77% identical to NTH1, was identified by the *S. cerevisiae* genome-sequencing project. Its cellular role is not known; neither its disruption nor its overexpression affects trehalose levels or the rate at which trehalose is degraded, and nth2 mutants display no loss of trehalase activity. Degradation of trehalose following heat shock is impaired to the same extent in nth2nth1 double mutants as in cells carrying the nth1 mutation alone. Regulation of Nth1p and Nth2p may, in part, be mediated by cAMP-dependent protein kinase; each protein has two potential sites for phosphorylation by this enzyme.

The expression of both **NTH1** and **NTH2** occurs constitutively, and increases at elevated temperatures.

### Box 1. Trehalose in historical perspective

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1681</td>
<td>The first recorded mention of trehala, the trehalose-containing cocoon for which the sugar was named. Produced by beetles of the genus <em>Larinus</em>, these cocoons are commonly found on the thistle-like plant <em>Echinops persicus</em>. The description appeared in the <em>Pharmacopoea Persica</em> by Friar Ange of Tolouse.</td>
</tr>
<tr>
<td>1832</td>
<td>While analyzing the constituents of ergot, a fungal infection of cereal grains, H. A. L. Wiggers identifies a sugar he calls ‘muttermolzucker’ (<em>ergot sugar</em>).</td>
</tr>
<tr>
<td>1858</td>
<td>Mitscherlich prepares the sugar described by Wiggers and names it mycose, from the Greek for fungus. M. Gubert describes trehala, one of the items in the Collection of Materia Medica contributed by M. Della Sudda of Constantinople to the 1855 Paris Universal Exposition. M. Berthelot analyzes the sugar contained in trehala, and names it trehalose. Berthelot notes the similarity of trehalose and mycose.</td>
</tr>
<tr>
<td>1873-1876</td>
<td>M. A. Muntz finds trehala in mushrooms and other fungi, but is unable to detect trehalose in yeast. He examines mycose and trehala and concludes that they are the same compound.</td>
</tr>
<tr>
<td>1893</td>
<td>E. Bourquelot prepares trehalose from Aspergillus niger.</td>
</tr>
<tr>
<td>1895</td>
<td>E. Fischer detects trehalase activity in yeast.</td>
</tr>
<tr>
<td>1925</td>
<td>E. Bourquelot prepares trehalose from Aspergillus niger.</td>
</tr>
<tr>
<td>1954</td>
<td>First successful chemical synthesis of α,α-trehalose, reported by R. U. Lemieux and H. F. Bauer.</td>
</tr>
</tbody>
</table>

Except where otherwise indicated, references to the original work can be found in Ref. 71.

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**Figure 2**

The resurrection plant *Selaginella lepidophylla*, which produces large quantities of trehalose, can sustain dramatic desiccation and still return to full activity upon rehydration. (Reproduced with permission of Quadrant Holdings.)
leading to the paradoxical situation that enzymes responsible for both trehalose synthesis and trehalose hydrolysis are further induced by heat (Table 1). Moreover, nth1 and nth2 mutants are actually impaired in their ability to survive and recover from severe heat (the double mutant even more so)\textsuperscript{32,37}, the opposite of what would be expected if trehalose protects cells. Work described below suggests an explanation for these surprising findings.

Trehalose as an energy reserve?

For more than half a century after yeasts were found to produce trehalose, the disaccharide was primarily thought to serve as a storage carbohydrate\textsuperscript{39–41}. This view was based on the observation that trehalose accumulates in physiological states in which energy storage is beneficial, such as stationary phase and sporulation. Correspondingly, trehalose is not produced when nutrients are abundant, as in exponential growth. The question remained, however, of why yeast requires a second energy store in addition to glycogen\textsuperscript{9,39}. Careful analysis of glycogen and trehalose accumulation in \textit{S. cerevisiae} sparked a dramatic change in the perception of how trehalose functions in yeast.

Energy reserves would be expected to accumulate when nutrients are abundant, in preparation for future scarcity\textsuperscript{9}. Glycogen is indeed produced during the exponential-growth phase of yeast cells, when ample glucose is available. By contrast, trehalose is not produced until glucose levels are nearly exhausted and the cells begin to enter stationary phase. Trehalose synthesis continues into stationary phase, in the absence of extracellular glucose, during which time glycogen is metabolized\textsuperscript{9}. Similarly, in sporulation, glycogen is utilized during the final maturation of ascospores; meanwhile, trehalose is not metabolized but is instead localized to the mature spores\textsuperscript{40}. During prolonged incubation in stationary phase, cells again utilize glycogen\textsuperscript{39}. Trehalose is ultimately also metabolized, but in what may be a desperate measure to avoid starvation: degradation of trehalose occurs long after glycogen stores are depleted and coincides with the onset of cell death\textsuperscript{9,39}. The different patterns of trehalose and glycogen accumulation and utilization suggested that these molecules play distinct roles in cellular physiology\textsuperscript{9}.

Trehalose as a stress protectant

A key contribution to understanding the function of trehalose in \textit{S. cerevisiae} was the finding that the disaccharide is localized exclusively to the nuclear-cytoplasmic compartment\textsuperscript{30}. Restriction of trehalose to this region (and its exclusion from other large spaces, such as the vacuole) results in cytosolic trehalose concentrations estimated at approximately 0.5 M\textsuperscript{42}, far greater than had previously been appreciated. Such high levels of the sugar, it was suggested, would dramatically affect the constituents of the cytoplasm\textsuperscript{42}. Indeed, high concentrations of trehalose have been shown in \textit{vivo} to extend the range of temperatures over which proteins retain their native state\textsuperscript{42,43}.

Table 1. Enzymes of trehalose metabolism in \textit{Saccharomyces cerevisiae}

<table>
<thead>
<tr>
<th>Trehalose synthesis</th>
<th>Properties</th>
</tr>
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<tbody>
<tr>
<td>Tps1p</td>
<td>Catalyses the synthesis of trehalose-6-phosphate from UDP–glucose and glucose-6-phosphate</td>
</tr>
<tr>
<td>Tps1p is expressed constitutively and is further induced by heat</td>
<td></td>
</tr>
<tr>
<td>tps1 mutants cannot grow on glucose-containing media and have impaired thermotolerance</td>
<td></td>
</tr>
<tr>
<td>Tps2p</td>
<td>Removes phosphate from trehalose-6-phosphate to yield trehalose</td>
</tr>
<tr>
<td>Tps2p is expressed constitutively and is further induced by heat</td>
<td></td>
</tr>
<tr>
<td>tps2 mutants have decreased survival of extreme heat</td>
<td></td>
</tr>
<tr>
<td>Tsl1p</td>
<td>Regulator of trehalose synthesis</td>
</tr>
<tr>
<td>Expression of Tsl1p increases during stationary phase</td>
<td></td>
</tr>
<tr>
<td>Tps3p</td>
<td>Regulator of trehalose synthesis</td>
</tr>
<tr>
<td>Expression of Tps3p is constant during exponential and stationary phase</td>
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<table>
<thead>
<tr>
<th>Trehalose degradation</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nth1p</td>
<td>Primary enzyme for trehalose degradation</td>
</tr>
<tr>
<td>Located in cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Nth1p is expressed constitutively and is further induced by heat</td>
<td></td>
</tr>
<tr>
<td>nth1 mutants have diminished thermotolerance and diminished recovery from heat shock</td>
<td></td>
</tr>
<tr>
<td>Nth2p</td>
<td>Highly homologous to Nth1p</td>
</tr>
<tr>
<td>Located to cytoplasm</td>
<td></td>
</tr>
<tr>
<td>Nth2 is expressed constitutively and is further induced by heat</td>
<td></td>
</tr>
<tr>
<td>nth2 mutants are impaired in thermotolerance and in recovery from heat shock</td>
<td></td>
</tr>
<tr>
<td>Ath1p</td>
<td>Located in vacuole</td>
</tr>
<tr>
<td>ath1 mutants are unable to utilize trehalose as a carbon source; mutants are reported to show enhanced survival of dehydration, freezing and ethanol stress</td>
<td></td>
</tr>
</tbody>
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Trehalose as a stress protectant

A key contribution to understanding the function of trehalose in \textit{S. cerevisiae} was the finding that the disaccharide is localized exclusively to the nuclear-cytoplasmic compartment\textsuperscript{40}. Restriction of trehalose to this region (and its exclusion from other large spaces, such as the vacule) results in cytosolic trehalose concentrations estimated at approximately 0.5 M\textsuperscript{42}, far greater than had previously been appreciated. Such high levels of the sugar, it was suggested, would dramatically affect the constituents of the cytoplasm\textsuperscript{42}. Indeed, high concentrations of trehalose have been shown in \textit{vivo} to extend the range of temperatures over which proteins retain their native state\textsuperscript{42,43}.

That these effects are important in stress tolerance \textit{in vitro} was demonstrated when trehalose levels were correlated with cell survival under adverse conditions. For
example, the physiological states under which trehalose levels are high – stationary phase and spores – are thermotolerant. In addition, trehalose concentrations increase as part of the cellular response to elevated temperatures, ethanol, desiccation and hydrogen peroxide. Observations in other fungi, prokaryotes and nematodes support the correlation in yeast that physiological circumstances in which there are high levels of trehalose coincide with increased tolerance to adverse environmental conditions.

The proposition that trehalose functions in tolerance was bolstered by the analysis of various S. cerevisiae mutations affecting trehalose levels. Cells carrying the hy1-1 mutation in the regulatory subunit of cAMP-dependent protein kinase are unable to synthesize high levels of trehalose and sensitive to heat. Conversely, cells

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Figure 3

Biosynthesis of trehalose. Saccharomyces cerevisiae produces trehalose in the cytosol through the sequential action of the Tps1p and Tps2p proteins; steps at which trehalose synthesis is blocked in the absence of these proteins are indicated. Tps1p and Tps2p associate physically to form the trehalose-synthase complex, together with either Tsl1p or Tps3p. The latter components appear to regulate the activity of the complex.
carrying the cyr1-2 mutation in the adenylate-cyclase structural gene produce trehalose constitutively and are thermotolerant. Interpretation of these results is necessarily circumscribed, however, because the same conditions that induce trehalose accumulation also affect the production of Hps, which, as noted above, are known to provide stress protection. The strongest evidence for an in vivo role for trehalose during heat shock emerged from the cloning of the TPS1 gene. Disruption of TPS1 prevents cells from synthesizing trehalose and severely compromises their thermotolerance. Yet this finding, too, is not unambiguous: a subsequent report indicated that these mutants are also impaired in Hps synthesis.

Three hypotheses have been proposed to explain the diminished thermotolerance of tps1 mutants: (1) the Tps1p protein, in addition to its enzymatic role in trehalose synthesis, is needed for the expression of heat-shock-protein genes—the thermosensitivity of tps1 mutants therefore results from a failure to induce the production of Hps fully; (2) trehalose only protects cells against mildly elevated temperatures, allowing the synthesis of Hps, which then enable cells to survive stress; and (3) a functional dichotomy exists in thermotolerance, in which trehalose acts directly to prevent protein denaturation at high temperatures, with those proteins that do denature being then bound by Hps and prevented from aggregating.

Yet other studies have engendered doubt as to whether trehalose synthesis plays any protective role at all. For example, after a heat pretreatment, cells grown in glucose produce more trehalose, yet are less thermotolerant than those grown in galactose. This observation in galactose, however, induces the synthesis of key Hps, which may well account for the difference in survival.

Thus, once again, interpretation of the effect of trehalose on thermotolerance is confounded by concomitant changes in Hps levels. Other observations are even more perplexing. The proposal that trehalose may have a protective role arose from the finding that, like Hps, trehalose is produced in response to stress. Yet trehalose is degraded soon after a stress has passed, far more rapidly than are Hps. This characteristic is enigmatic, considering that even high concentrations of trehalose do not interfere with the stability of denatured proteins. Indeed, the trehalose-stabilized denatured substrate showed elevated ANS (1-anilino-naphthalene-8-sulphonate) fluorescence. High levels of ANS fluorescence are observed when the fluorophore interacts with partially folded proteins displaying elements of secondary structure, but lacking tertiary structure. Indeed, the trehalose-stabilized denatured substrate showed elevated ANS fluorescence.

This series of experiments also suggests why trehalose must be degraded rapidly after heat shock: if the luciferase is subsequently dialuted out of trehalose, it can then be efficiently refolded by molecular chaperones; however, if concentrations of trehalose remain high during the in vivo refolding reaction, they interfere with luciferase reactivation. A similar phenomenon was observed in vitro reactivation of a heat-denatured reporter protein was impaired in nth1 mutants, which cannot efficiently hydrolyze accumulated trehalose during recovery from heat shock.

The observations now fit together to form a coherent explanation of the role of trehalose in thermotolerance and of its interplay with Hps in that process (Fig. 4). Both mechanisms are critical in S. cerevisiae for optimal survival of heat shock.

Trehalose prevents native proteins from denaturing at high temperatures. Those proteins that do unfold are bound by Hps, which suppress their aggregation and promote their proper refolding. Trehalose can also stabilize those substrates and thereby reduce aggregation when the protein-repair machinery is overwhelmed. However, the stabilization of denatured proteins by trehalose can interfere with their subsequent refolding and, unless trehalose is rapidly degraded after heat shock, the disaccharide can impede the ability of viability, metabolic labelling and western-blot analysis demonstrated no difference in the production or accumulation of Hps in wild-type and tps1 mutants. Under such conditions, tps1 mutants were still greatly impaired in thermotolerance. Genetic experiments also pointed to a distinct role for trehalose and Hps. In these experiments, tps1 mutants were engineered to lack Hps1-104, as well as the TPS1 gene. In both cases, the acquisition of thermotolerance by the double mutant was impaired far more than that of either single mutant.
Hsps to resolve heat-induced damage to cellular proteins swiftly.

Potential applications

The protein-stabilization capacities of trehalose, elucidated by many laboratories, suggest a number of potential uses. These include enhancing the stress tolerance of commercially important organisms, facilitating the production of recombinant proteins and, in the long term, treating disorders resulting from protein instability and aggregation.

The ability to manipulate stress tolerance is desirable for a variety of agricultural and industrial uses. Efforts have already been made to increase trehalose levels in

Figure 4

(a) High temperatures cause proteins to denature. Unfolding proteins can then associate to form aggregates. (b) Saccharomyces cerevisiae synthesizes large quantities of trehalose during heat shock. The disaccharide stabilizes proteins in their native state under these conditions, and also suppresses aggregation of proteins that have denatured. (c) Yeast cells normally degrade trehalose rapidly after heat shock. During recovery, molecular chaperones promote the reactivation of denatured substrates that have been prevented from aggregating. (d) The persistence of high levels of trehalose interferes with the reactivation of denatured substrates, explaining both the need to degrade trehalose during recovery and the thermosensitivity of mutants unable to do so.
plant, both to heighten tolerance and to produce large quantities of the sugar inexpensively for industrial purposes.4,5 The emerging appreciation of the distinct roles of trehalose and Hsps now suggests that the recombination of the former goal will probably require the coexpression of Hsps for optimal results. Genetic manipulation of the trehalase-degrading enzymes in yeast may contribute to various aspects of baking and brewing; for example, as high levels of trehalose are correlated with enhanced freeze tolerance, baker’s yeast used in the preparation of frozen doughs is routinely optimized for maximal trehalose content. The production of dough initiates fermentation, however, which, in turn, leads to the degradation of trehalose; disruption of the trehalase genes may circumvent this problem.6 Similarly, the elevated ethanol tolerance of strains mutants may enable them to overcome current limitations on the alcohol levels of wine or beer, which result from ethanol toxicity to the yeast carrying out the fermentation.7 More significantly, such mutants may also serve in the production of ethanol as an alternative energy source.8

The ability of trehalose to suppress aggregation might be used to advantage in the commercial production of heterologous proteins expressed in E. coli, which itself synthesizes trehalose in response to stress. Such proteins frequently aggregate into inclusion bodies. The overexpression of the bacterial chaperones GroEL and GroES has already been shown to be of some help in preventing inclusions,9 but the general promise that chaperones were thought to hold has not been realized. The production of functional recombinant proteins may instead require the overproduction of trehalose together with chaperones. Even if substrate proteins are only prevented from aggregating, it may be possible to induce them then to fold properly through the careful adjustment of the conditions following lysis.

Finally, organic solutes have shown striking effects in connection with proteins involved in important human diseases. One such malady is the neurodegenerative disorder Creutzfeld-Jakob disease (CJD). Like its more pathological form, Creutzfeld–Jakob disease (CJD). Like its more human counterpart, protein that causes the disease. In both cases, the exogenous addition of organic solutes to tissue-culture cells expressing these substrates has prevented the proteins from adopting conformations associated with their respective diseases.10 Based on these results, it has been suggested that small organic molecules may be useful as therapeutic agents in the treatment of conditions resulting from proteins whose native state is unstable, either naturally or because of mutations.11 The ability of trehalose and other solutes to suppress aggregation suggests that they may also be employed against illnesses now known to be caused by aggregation, including amyloid disorders and, most recently, Huntington’s disease.6,12,13 Of course, technical obstacles ranging from production and delivery to potential interference with normal protein-folding pathways need to be surmounted before such strategies become practical.

Some of the most fundamental aspects of trehalose biology, such as its regulation, still remain to be elucidated. Nevertheless, as our understanding of the complex balance of beneficial and detrimental effects of this unusual sugar continues to grow, the prospects for harnessing its properties for promising biological and commercial applications become ever brighter.

Acknowledgments

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References

Plastic antibodies: developments and applications
Karsten Haupt and Klaus Mosbach

Antibodies are natural receptor molecules produced after contact with an antigen. In an attempt to mimic nature, the technique of molecular imprinting has been developed, which allows specific recognition sites to be formed in synthetic polymers through the use of templates. These recognition sites mimic the binding sites of antibodies and may be substituted for them in applications such as affinity separation, assay systems and biosensors. The stability and low cost of these polymers make them particularly attractive to industry.

Antibodies are routinely utilized as analytical reagents in clinical and research laboratories. Two of their most common applications are in immunosassays and immunoaffinity separations, but interest is also increasing in their use in immunosensors. A common denominator in all of these techniques is the binding of an analyte to an antibody. The binding utilizes the exquisite recognition properties of an antibody for the antigen, in which the antigen fits exactly into the antibody's binding site, whereas other, even structurally related, compounds are excluded from the site.

Researchers have attempted to replace antibodies with smaller, more stable counterparts, and have also been searching for alternative ways to obtain antibody-like receptors. This has led, for instance, to the development of minibodies and diabodies, which are biologically active fragments of antibodies but still retain the ability to bind antigen with smaller, more stable counterparts, and have also been searching for alternative ways to obtain antibody-like receptors. This has led, for instance, to the development of minibodies and diabodies, which are biologically active fragments of antibodies but still retain the ability to bind antigen. They can be produced either by chemical synthesis or through recombinant DNA technology.

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