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Activity of the α-glucoside transporter Agt1 in *Saccharomyces cerevisiae* cells during dehydration-rehydration events

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ABSTRACT

Microbial cells can enter a state of anhydrobiosis under desiccating conditions. One of the main determinants of viability during dehydration-rehydration cycles is structural integrity of the plasma membrane. Whereas much is known about phase transitions of the lipid bilayer, there is a paucity of information on changes in activity of plasma membrane proteins during dehydration-rehydration events. We selected the α-glucoside transporter Agt1 to gain insights into stress mechanisms/responses and ecophysiology during anhydrobiosis. As intracellular water content of *S. cerevisiae* strain 14 (a strain with moderate tolerance to dehydration-rehydration) was reduced to 1.5 g water/g dry weight, the activity of the Agt1 transporter decreased by 10–15 %. This indicates that functionality of this trans-membrane and relatively hydrophobic protein depends on water. Notably, however, levels of cell viability were retained. Prior incubation in the stress protectant xylitol increased stability of the plasma membrane but not Agt1. Studies were carried out using a comparator yeast which was highly resistant to dehydration-rehydration (*S. cerevisiae* strain 77). By contrast to *S. cerevisiae* strain 14, there was no significant reduction of Agt1 activity in *S. cerevisiae* strain 77 cells. These findings have implications for the ecophysiology of *S. cerevisiae* strains in natural and industrial systems.

1. Introduction

Yeasts, like the majority of microbes, can be exposed to dehydration-rehydration cycles as solute concentrations, temperature, evaporation and other parameters and events modify the water activity of the microbial habitat. Both the spores and vegetative cells of microbes can curtail their metabolism and enter a state of anhydrobiosis when the amount of intracellular water falls below a critical threshold (Morgan et al., 2006; Garcia, 2011; Dupont et al., 2014; Wyatt et al., 2015a; Rapoport et al., 2016; Rapoport, 2017; Esbelin et al., 2018). This phenomenon was first described more than 300 y ago (Leeuwenhoek 1705), but various aspects of the process remain unresolved. Studies of anhydrobiosis have been carried out to characterise key structural and functional changes within yeast and fungal cells during dehydration-rehydration cycles, metabolic reactivation upon rehydration, and mechanistic aspects involved in the preservation of cell viability (Guzhova et al., 2008; Borovikova et al., 2014b; Dupont et al., 2014; Rapoport et al., 2014, 2016; Rapoport, 2017). The microbial cell membrane is highly vulnerable to environmental challenges, so modifications of membrane composition represents a primary mechanism of adaptation and survival in relation to diverse types of stress (e.g. Crowe et al., 1984; McCammick et al., 2010; Santos et al., 2015). Invaginations of the plasma membrane can be clearly observed in dehydrated yeast cells using electron microscopy (Rapoport, 2017). In *Saccharomyces cerevisiae* cells subjected to dehydration-rehydration cycles, there is increased membrane permeability (Rapoport et al., 1995, 1997; Rapoport, 2017) and phase transitions of the lipid bilayer (Crowe et al., 1984, 1989; Dupont et al., 2014; Rapoport, 2017). A model, developed to illustrate changes in molecular organization of cellular membranes, has successfully predicted the actual plasma membrane changes which

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take place under their desiccation, in taxonomically diverse systems (Crowe et al., 1989). Compounds such as trehalose, trehalose-based oligosaccharides, and sugar alcohols (mannitol, inositol, xylitol, glycerol and others) can be synthesised by cells of yeasts and fungi during dehydrating or desiccating conditions, and act to protect membranes and other macromolecular systems (Crowe et al., 1984; Rapoport et al., 1988, 2009; Alves et al., 2015; Wyatt et al., 2015b; Stevenson et al., 2017a). According to the recently modified 'water-replacement hypothesis', it can be predicted that these compounds stabilise dry membranes by preventing a decrease in the spacing between the membrane lipids and cause a concentration-dependent increase of the area per lipid (APL) that is accompanied by increased fluidity of the lipid bilayer (Crowe et al., 1984, 1992; Golovina et al., 2009, 2010; Dupont et al., 2014). This activity arises, in part, from the kosmotropicity of trehalose, trehalose-based oligosaccharides, and/or some polyols (sugar alcohols) (Cray et al., 2013a, 2016; Wyatt et al., 2015a). In addition, ergosterol affords mechanical reinforcement of membranes and antioxidative protection of lipids in conditions of dehydration-rehydration (Dupont et al., 2014; Rapoport, 2017).

The biophysical changes in the lipid bilayer portions of the yeast cell membrane have been relatively well documented, yet there is little known about the potential role of plasma membrane proteins *in vivo* during dehydration-rehydration events. The current study was carried out to assess activity of a plasma membrane protein which is involved in maltose accumulation in *S. cerevisiae*, α-glucoside transporter Agt1, and is used here as a model system to gain insights into stress mechanisms/responses and the ecophysiology of this yeast during anhydrobiosis.

2. Materials and methods

2.1. Yeast strains and culture conditions

The yeast strains *S. cerevisiae* strain 14 and *S. cerevisiae* strain 77 obtained from the Microbial Strain Collection of Latvia (http://mikro.daba.lv) were grown in flasks with yeast extract peptone maltose broth (YPMB) containing 10 g l^{-1} yeast extract, 20 g l^{-1} peptone and 20 g l^{-1} maltose with initial pH 5.0, at 180 rpm using Orbital Shaker-Incubator ES-20/60 (Biosan, Latvia) and 30 °C for 16 h (to exponential growth phase) and 42 h (to the mid of stationary growth phase). Cells were harvested by centrifugation, washed twice with distilled water and used for (i) quantitation of Agt1 activity, (ii) exposure to dehydration stress and subsequent measurement of Agt1 activity and (iii) incubation in 1M xylitol solution, exposure to dehydration stress and subsequent measurement of Agt1 activity.

2.2. Incubation of the yeast in 1M xylitol solution

Centrifuged pellets of both strains of yeast *S. cerevisiae* biomass (3 g) were placed into 200 ml of 1 M xylitol solution and incubated on a rotary shaker (30 °C) for 3 h. Thereafter, the yeast biomass was harvested by centrifugation for 10 min at 2600 g at room temperature (22 °C) for its further dehydration or measurement of Agt1 activity.

2.3. Dehydration and rehydration procedures

Yeast biomass sieved (1-mm mesh diameter), spread in a 1-mm layer in the Petri dish and then subjected to convective drying in a fan-free oven at 30 °C for 12 h (standard dehydration) or 16 h (prolonged dehydration). The minimum time needed to cells to reach 0.085–0.100 g water/g dry weight (indicating that anhydrobiosis had been reached) was 12 h. 0.05 g of dehydrated biomass was rehydrated using rapid or gradual rehydration. Rapid rehydration was performed in a tube containing distilled water (5 ml) for 10 min at room temperature (−20 °C).

Gradual rehydration was performed in a glass, water-vapour chamber at 37 °C for 2 h. A Petri dish with 0.05 g of dehydrated biomass was placed in the middle part of the chamber, and water was poured in its bottom part. After gradual rehydration by the water vapour, rapid rehydration of partially rehydrated yeast cells was performed by placing cells directly into distilled water (5 ml) for 10 min. A temperature 37 °C was optimal for maintaining viability during slow hydration, according to our previous studies (e.g. Beker et al., 1984).

2.4. Determination of the viability of yeast after dehydration

The viability of dehydrated yeast samples was checked after 10 min rehydration in distilled water at room temperature using fluorescent microscopy and a fluorochrome primulin (Rapoport and Meysel, 1985).

2.5. Determination of cellular water content

The water content of dehydrated cells was determined by drying the biomass at 105 °C until a constant weight was reached. The differences of the sample weights before and after drying were calculated to determine water loss, which was indicative of water content of the original sample (Rapoport et al., 1995).

2.6. Cell membrane permeability

The changes of cell membrane permeability during dehydration/rehydration stress were quantified by calculating percentage of the difference of yeast dry weight before and after rehydration.

2.7. Investigation of Agt1 activity

Yeast cell suspensions were prepared with 0.2 OD_{600} inoculum and their dry weights (5 ± 0.2 mg/ml) determined by drying a fixed volume of cell suspension at 105 °C until a constant weight was reached. Activity of the Agt1 transporter was measured as follows: 20 μl of yeast cell suspension was suspended in 160 μl of potassium phosphate buffer (pH 5.0) and incubated for 5 min at 30 °C. Then, 20 μl of *p*-nitrophenyl-α-glucoside (pNPαG) (40 mM solution in distilled water) was added as a substrate for the Agt1 transporter. After timed intervals, aliquots were removed and heated in a thermoblock at 100 °C for 3 min to stop the reaction. The samples were then cooled on ice, 1 ml of 2.0M $NaHCO_3$ (pH 10.0) was added to enhance the colour reaction of the samples, and the yeast cells were removed from the sample by centrifugation. The quantity of p-nitrophenol (p-NP) in the supernatant of the cell suspension was determined at 400 nm using a spectrophotometer (Ultrospec 2100 pro; Amersham Biosciences). Preboiled cells were used as blanks in control experiments. A calibration curve for p-NP was made to relate the optical values to concentrations (Stambuk, 2000). The amount of produced p-NP per min was normalized to yeast dry weight (p-NP, mM/mg dry weight).

2.8. Determination of maltase activity in yeast cell extracts

Yeast cells for preparation of extracts were suspended in 100 mM K-phosphate buffer (pH 6.5) containing 0.1 mM EDTA (maltase buffer). Yeast cells were disintegrated by Mini-Beadbeater at 5 × 10^3 rpm for 15 min using glass beads diam. 0.5 mm in ratio 1:1. Supernatants obtained after the centrifugation (20 min at 16 162 g at 4 °C) were used as crude cell-free extracts. Maltase

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