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Solubilization and characterization of a cell wall-bound trehalase from ascospores of the fission yeast *Schizosaccharomyces pombe*

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Summary

The genome of the fission yeast *Schizosaccharomyces pombe* lacks sequence homologs to *ath1* genes coding for acid trehalases in other yeasts or filamentous fungi. However, acid trehalase activity is present at the spore stage in the life cycle of the fission yeast. The enzyme responsible for this activity behaves as a surface enzyme covalently linked to the spore cell walls in both wild-type and *ntp1* mutant strains devoid of neutral trehalase. Lytic treatment of particulated cell wall fractions allowed the solubilization of the enzyme into an active form. We have characterized this soluble enzyme and found that its kinetic parameters, optimum pH and temperature, thermal denaturation and salt responses are closely similar to other conventional acid trehalases. Hence, this rather unusual enzyme can be recognized as acid trehalase by its biochemical properties although it does not share genetic homology with other known acid trehalases. The potential role of such acid trehalase in the mobilization of trehalose is discussed.

Introduction

Trehalose plays a key physiological role in yeast cells as reserve and stress metabolite (van Laere, 1989). Enzymes that breakdown this storage disaccharide are specific hydrolases known as trehalases. Yeast trehalases are classified in two classes, neutral (regulatory) and acid (non regulatory) trehalases, on the bases of their optimum pH for activity, ability to become activated by covalent modification and localization into the cells (Thevelein, 1984). Neutral trehalases coexist with trehalose within the cytosol as cryptic enzymes whose activity can be triggered by cAMP-dependent phosphorylation of the enzyme protein. Acid trehalases are compartmentalized into vacuoles or at the cell surface and their activity is not regulated by post-traslational

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mechanisms (Thevelein, 1984). Recently, it has been suggested to rename neutral and acid trehalases as cytosolic and extracellular trehalases, respectively, to describe more adequately their localization and function in the yeast cells (Parrou et al., 2005). Trehalases with mixed biochemical properties have been described in thermophilic fungi (Lucio-Eterovic et al., 2005).

The implication of neutral trehalases in the hydrolysis of endogenous trehalose is well documented (Cansado et al., 1998). Besides, it is generally accepted that the main function of acid trehalases is related to the utilization of exogenous trehalose as carbon source (d'Enfert and Fontaine, 1997; Parrou et al., 2005). However, the involvement of acid trehalases in the catabolism of intracellular trehalose has been suggested in several reports. In particular, Inoue and Shimoda (1981) proposed that the mobilization of endogenous trehalose in the fission yeast *Schizosaccharomyces pombe* during spore germination might be carried out by a cell wall-bound acid trehalase. They also suggested that the germination stimuli would alter plasma membrane permeability to allow the cytosolic trehalose to reach the trehalase enzyme located outside the spore. Later work demonstrated that in addition to the sporulation-specific acid trehalase, a cytosolic neutral trehalase exists in the vegetative cells of the fission yeast, leading to the assumption that such enzyme would be responsible for the mobilization in spores of the stored trehalose (De Virgilio et al., 1991). However, subsequent studies described that trehalose was still mobilized during germination in spores from *Sch. pombe* mutants defective in neutral trehalase, although at a much lower rate than in wild type spores (Beltran et al., 2000). Moreover, inhibition of the acid trehalase in these mutants blocked trehalose catabolism and greatly retarded spore germination, suggesting an important role in the breakdown of this reserve carbohydrate during the germination process (Beltran et al., 2000). The decrease in trehalose content observed in the absence of neutral enzyme was attributed to the acid trehalase, which was thus considered to participate in the hydrolysis of the endogenous trehalose as an ancillary enzyme. In contrast to all biochemical data supporting the presence of an acid trehalase activity during sporulation, the complete sequencing of the *Sch. pombe* genome revealed the absence of sequence homologs to known acid trehalases from other yeasts and fungi (Parrou et al., 2005). This unexpected finding has hampered direct genetic approaches in studies on the nature and physiological significance of the acid trehalase of *Sch. pombe*. In view of these conflicting results, we reinvestigated the trehalase enzyme of the fission yeast spores to present additional data related to its localization, solubilization and biochemical characterization.

Materials and methods

Cell strains, culture and ascospore isolation

Sch. pombe strain 968 (h^{90} *ura4-D18*) and strain MMT48 (h^{90} *ura4-D18 ntp1:: ura4*$^+$), which lacks neutral trehalase activity, were used in this study (Beltran et al., 2000). To obtain spores, cells were pre-grown until mid-exponential phase in YES liquid medium containing 2% glucose plus 0.6% yeast extract, washed in sterile distilled water and further incubated for 6 days in liquid MEL sporulation medium, which contained 3% malt extract in 50 mM sodium phosphate buffer pH 5.9 (Gutz et al., 1974). For ascospore isolation, samples of sporulated cultures were subjected to isopycnic centrifugation on linear density gradients of urografin (25–55%, v/v) in a swinging-bucket rotor at 25,000*g* for 30 min and 4 °C (Nishi et al., 1978). The samples resulted partitioned after centrifugation in three distinct isolated bands that were analyzed by phase-contrast microscopy. The upper fraction contained cellular debris and unbroken asci while the intermediate fraction was composed of remaining whole vegetative cells. Isolated intact ascospores were located purified in the band of more density (45–50% urografin), extensively washed with distilled water at low-speed centrifugation, resuspended in assay buffer and directly used as enzyme source in most experiments.

Treatment of spores, preparation of cells extracts and cell walls

Mild acid treatments of spores were performed essentially as indicated by Arnold (1972). Briefly, spores were subjected to 1-h treatments at room temperature in 0.1 N HCl or 3 N H_3PO_4 and then exhaustively washed on the centrifuge with 50 mM acetate buffer, pH 4.6. The percentage of live cells was ascertained by the methylene blue test in conjunction with a hemocytometer (Arnold, 1972). In parallel, spore viability was determined by the plate forming units method. Cell-free extracts from treated spores were prepared by mechanical disruption with glass beads as described previously (Carrillo et al., 1992) and tested for trehalase and marker enzymes against cell extracts from