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Evidence for Nonregulatory Trehalase Activity in *Dictyostelium discoideum*

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Abstract. An in vitro activation treatment, stimulatory to the regulatory cytoplasmic trehalase of *Saccharomyces cerevisiae*, had no effect on the lysosomal trehalase of *Dictyostelium discoideum*. Concentrations of cAMP that produced a 19 to 22-fold increase in trehalase activity in *S. cerevisiae* extracts did not stimulate trehalase activity in *D. discoideum* extracts. cGMP and 5′-AMP were also not effective in activating the enzyme. *Dictyostelium discoideum* trehalase exhibits characteristics typical of nonregulatory trehalases, in agreement with its lysosomal localization. The data are consistent with the hypothesis that changes in compartmentation regulate trehalose mobilization in *D. discoideum*.

The control of trehalose breakdown in fungi is of interest because of the potential role of this disaccharide as a source of energy during development. In fungi, the only enzyme capable of hydrolyzing trehalose is trehalase (EC 3.21.1.28) [21]. The stable existence of cytoplasmic trehalase and trehalose in sporulating *Saccharomyces cerevisiae* and several other fungi may result from the facile interconversion of a phosphorylated (active) trehalase into a dephosphorylated (inactive) trehalase. These fungi are said to possess regulatory trehalases [21]. In addition, *S. cerevisiae* has a nonregulatory vacuolar trehalase [18], which is a lysosomal enzyme [13]. It has been postulated that regulation of nonregulatory trehalases is mediated by spatial separation of the enzyme from its substrate [14, 16, 21].

Trehalase from *Dictyostelium discoideum* is similar to seven other lysosomal enzymes because of its acidic pH optimum [5] and its presence at high specific activity in the matrix material of fruiting bodies [6, 15, 20]. Several lines of evidence indicate trehalase to be a lysosomal enzyme in *D. discoideum*. First, particulate fractions of vegetative cells contained trehalase during homogenization only when sucrose was present as an osmotic protectant. Furthermore, the presence of the common lysosomal antigenic determinant on trehalase has been established [20]. The altered characteristics of trehalase in strain M31, which is deficient in the oligosaccharide-processing enzyme, α-1,3-glucosidase [12], provides additional proof of the lysosomal nature of *D. discoideum* trehalase [manuscript in preparation].

The present study utilizes *S. cerevisiae* as a control system and in vitro conditions known to activate the regulatory trehalase of *S. cerevisiae* to demonstrate the nonregulatory nature of trehalase from *D. discoideum*.

Materials and Methods

Organism and culture conditions. *Dictyostelium discoideum* strain AX3 was grown in TM medium [11]. Strain NC4H was grown in conjunction with *Escherichia coli* B/r on glucose–salts agar [1, 9]. *Saccharomyces cerevisiae* strain X2180 (Yeast Genetic Stock Centre, Berkeley, California) was grown in YEPD broth [13].

Preparation of crude enzyme from *D. discoideum*. The cells were harvested when titers of 1–1.5 × 10^7 were reached. They were washed by centrifugation at 4000 rpm for 10 min at 2°C in a Beckman refrigerated centrifuge. The pelleted cells were resuspended in cold phosphate buffer (10 m*M*, pH 6.0) and centrifuged again. This procedure was repeated at least twice. The pellet was finally resuspended in the buffer and passed through a French Pressure cell three times at 20,000 psi (1.3 × 10^5 kPa). The extract was centrifuged at 27,000 *g* for 30 min in a Beckman J-21 centrifuge at 4°C. The supernatant fraction served as the endogenous trehalase preparation.

When crude enzyme from the *D. discoideum* spores had to be isolated, then 20 plates of spores were harvested. The spores were washed at least thrice to eliminate contamination from the

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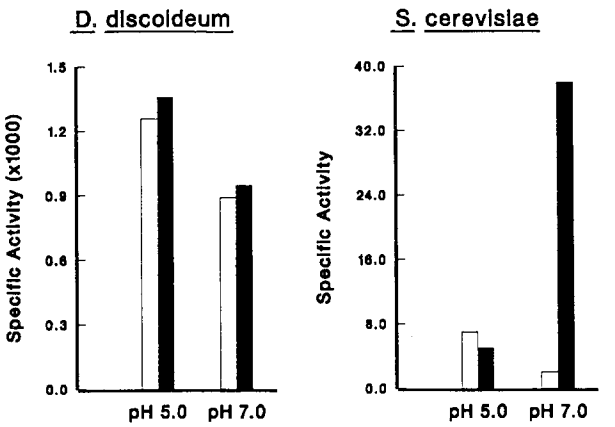

Fig. 1. Effect of 10 μM cAMP on trehalase activity from *Dictyostelium discoideum* and *Saccharomyces cerevisiae*. Values are the means; SEM ± 10%. Specific activity of trehalase in the absence (□) and presence (■) of activation treatment.

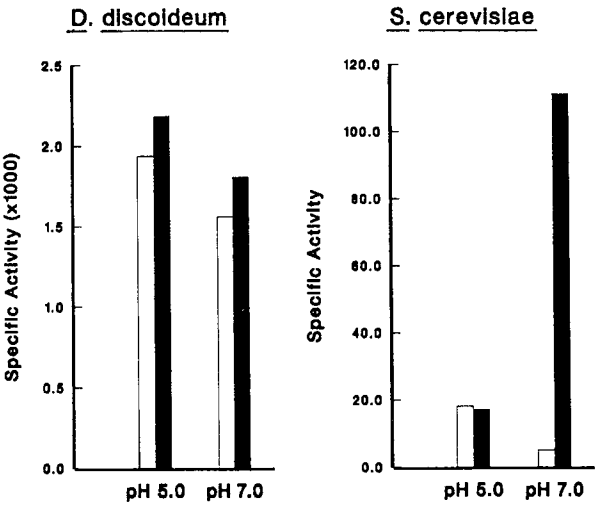

Fig. 2. Effect of 20 μM cAMP on trehalase activity from *Dictyostelium discoideum* and *Saccharomyces cerevisiae*. Values are the means; SEM ± 2.5%. Specific activity of trehalase in the absence (□) and presence (■) of activation treatment.

enzyme derived from the matrix, passed through a French Pressure cell, and processed as described above.

Preparation of crude extracts from *S. cerevisiae*. Mid-exponential growth-phase cells were harvested by centrifugation at setting 6 in an IEC clinical tabletop centrifuge. The cells were washed once and were resuspended in 5 ml of sterile, double-distilled water. The cells were disrupted by three passages through a French Pressure cell at 20,000 psi (1.3×10^5 kPa). The extract was centrifuged at 18,000 *g* for 15 min in a Beckman J-21 centrifuge at 4°C. The supernatant was used as the crude cell extract.

In vitro activation of trehalase. The activation treatment was based on the method of Ortiz et al. [19]. The activation mixture consisted of 4 m*M* adenosine triphosphate (ATP), 9 m*M* magnesium sulfate, 50 m*M* sodium fluoride, 5 m*M* theophylline, and one of the following: 50 μM or 100 μM cyclic adenosine monophosphate (cAMP) or 5'-adenosine monophosphate (5'AMP). The activation mixture was prepared in 0.05 *M* phosphate buffer, pH 7.5; 0.1 ml of the activation mixture was added to 0.4 ml of the extract. The control received 0.1 ml of the buffer. The reaction mixture was incubated at 35°C for 10 min and interrupted by adding 1.5 ml of 0.07 *M* maleate buffer, pH 6.0, containing 10 m*M* EDTA. The mixture was then centrifuged at low speed, and the supernatant was used to determine trehalase activity.

Trehalase enzyme assay. Trehalase was quantified with trehalose as the substrate and a discontinuous, coupled glucosidase assay [7]. The buffers used were those of Londesborough and Varimo [18]. Vacuolar trehalase activity was measured in 50 m*M* MES buffer, pH 5.0, with 0.1 m*M* EDTA. The cytoplasmic trehalase of *S. cerevisiae* was measured in 50 m*M* PIPES buffer, pH 7.0. Specific activity is defined as nanomoles of glucose released per minute per mg protein.

Protein determination. Protein was measured by the Bradford method [3], with rabbit α-globulin.

Results

The *Saccharomyces cerevisiae* (vacuolar, pH 5.0 optimum) trehalase incubated in the presence of 10 μM cAMP showed no increase in activity over the control (Fig. 1). The cytoplasmic enzyme (pH 7.0 optimum) showed a 19-fold increase in activity when the sample was incubated in the presence of the activation mixture. The *Dictyostelium discoideum* enzyme showed no increase in activity in the presence of 10 μM cAMP (Fig. 1). The slight drop in activity observed at pH 7.0 is a result of the fact that the lysosomal trehalase of *D. discoideum* has a pH optimum of 5.5 [5].

Similar results were obtained when the activation treatment employed 20 μM cAMP (Fig. 2). The cytoplasmic enzyme activity of *S. cerevisiae* increased 22-fold compared with the control. The *D. discoideum* trehalase once again was not significantly activated. Activation of trehalase was attempted in the presence of 10 μM 5'-AMP (Fig. 3) and 10 μM cGMP (Fig. 4). Trehalases from *D. discoideum* and *S. cerevisiae* were not activated under these conditions. The dormant spores of *D. discoideum* contain a basal level of trehalase activity that is utilized to mobilize trehalose during early spore germination [14]. The data of Fig. 5 indicate that cAMP at concentrations of 20 μM and 40 μM did not stimulate spore trehalase activity.

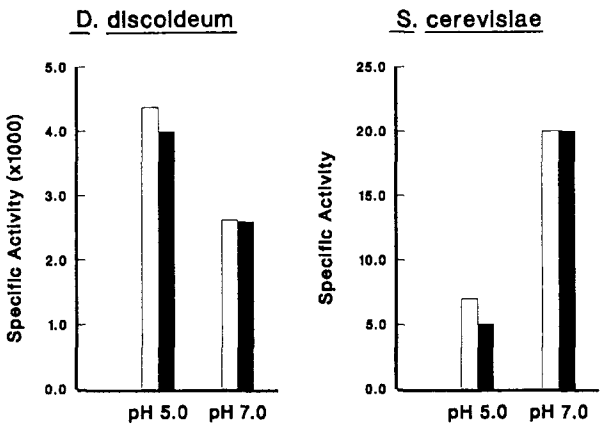

Fig. 3. Effect of 10 μM 5'AMP on trehalase activity from *Dictyostelium discoideum* and *Saccharomyces cerevisiae*. Values are the means; SEM ± 8%. Specific activity of trehalase in the absence (□) and presence (■) of activation treatment.

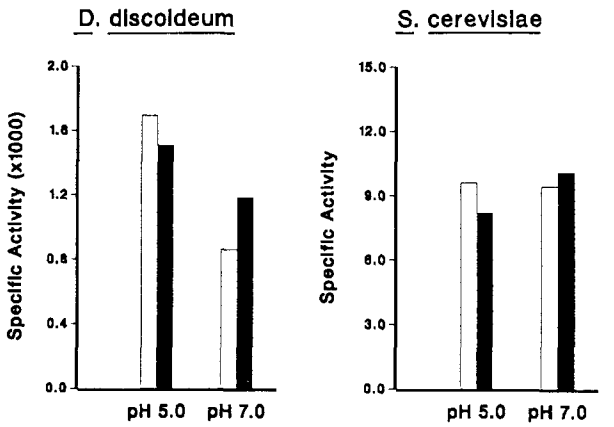

Fig. 4. Effect of 10 μM cGMP on trehalase activity from *Dictyostelium discoideum* and *Saccharomyces cerevisiae*. Values are the means; SEM ± 9%. Specific activity of trehalase in the absence (□) and presence (■) of activation treatment.

Discussion

It has been reported by several authors that addition of glucose, uncouplers, and nystatin cause trehalase activation in *S. cerevisiae* [21]. These agents cause an elevation in the intracellular levels of cAMP, which activates a cAMP-dependent protein kinase. Trehalase, which is a substrate for this kinase [24], is in turn activated. Initially it was believed that membrane depolarization was the mediating factor for trehalase activation, but recent evidence [4, 25] attributes internal acidification as the cause for activation.

Evidence for activation of trehalase under in vitro conditions by cAMP-dependent protein phosphorylation has been presented for *S. cerevisiae* [18, 19, 22, 26], *Candida utilis* [2], and *Phycomyces blakesleeanus* [27]. Concentrations of cAMP that produced a 19- to 22-fold increase in the cytoplasmic trehalase activity of *S. cerevisiae* did not stimulate trehalase activity in *D. discoideum*; cGMP and 5'-AMP were also not effective in activating the enzyme. Thevelein [22] reported that cGMP was also effective in activation of trehalase from vegetative cells of *S. cerevisiae* under in vitro conditions. However, the level of cGMP in vivo was tenfold lower than the cAMP level, and in vitro activation was observed only at 10-fold higher concentrations of cGMP. It was, therefore, considered unlikely for cGMP to be involved in the regulation of trehalase.

The trehalase from *D. discoideum* has an acid pH optimum [5] and high heat stability [17]. These characteristics fit with the purely hydrolytic nature

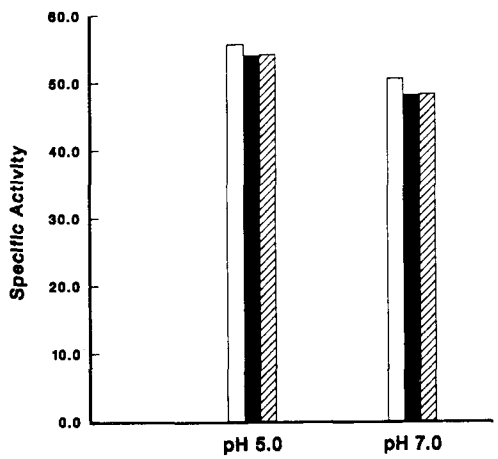

Fig. 5. Effect of 20 μM and 40 μM cAMP on trehalase activity from dormant spores of *Dictyostelium discoideum*. Values are the means; SEM ± 5.5%. Specific activity of trehalase in the absence (□) and presence of 20 μM cAMP (■) and 40 μM cAMP (▨) in the activation treatment.

of the enzyme. Saltatory activity changes of trehalase during periods of rapid trehalose mobilization have never been observed in *D. discoideum*. For example, in spore germination, trehalose is rapidly hydrolyzed during early spore swelling [14], but no increase in trehalase activity is observed at this stage. The burst of trehalase synthesis occurs much later, i.e., prior to emergence [8, 14]. This increase in activity requires both RNA and protein synthesis [9, 10, 23].

To date no evidence has been obtained for in vitro activation of nonregulatory trehalases by pro-

tein phosphorylation. The present study demonstrates that *D. discoideum* trehalase cannot be activated under conditions known to activate other regulatory trehalases. In having the above-mentioned characteristics, trehalase from *D. discoideum* meets all the requirements of being a so-called nonregulatory trehalase. The nonregulatory nature of *D. discoideum* trehalase, coupled with its lysosomal location, implicates compartmentation to be the phenomenon mediating trehalose utilization.

ACKNOWLEDGMENTS

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