Does a cyclic AMP-dependent phosphorylation initiate the transfer of trehalase from the cytosol into the vacuoles in *Saccharomyces cerevisiae*?

Andres Wiemken and Maja Schellenberg

Institut für Allgemeine Botanik ETH, Sonneggstr. 5, CH-8092 Zürich, Switzerland

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Trehalase activity in a yeast protoplast lysate increased ~40-times upon preincubation with cAMP and ATP. The activity present without the preincubation could all be sedimentated at 8000 × *g*, for 10 min confirming the previously reported localization of the active trehalase (Ta) in the vacuoles. Virtually all the trehalase activity newly formed upon the preincubation, however, was found in the soluble fraction, indicating that a trehelase-zymogen (Tz) is located in the cytosol. This raises the possibility that a cAMP-dependent phosphorylation not only transforms Tz to Ta but also initiates the transfer of trehalase from the cytosol into the vacuoles.

Yeast *Trehalase* *Vacuole* *Lysosome* *Cytosol* *cAMP*

1. INTRODUCTION

Under certain conditions, for instance after induction of spore germination [1] or initiation of growth in a stationary culture [2], trehalase activity in yeast increases greatly and almost instantaneously. This appears to involve activation of a preexisting trehalase-zymogen (Tz) by a cAMP-dependent protein kinase [3]. Having shown that the active trehalase (Ta) is located in the vacuoles [4] it interested us to see where the zymogen (Tz) is located.

2.. MATERIALS AND METHODS

Saccharomyces cerevisiae (strain C 276 a/α, obtained from Dr J. Pringle; for trehalase metabolism, see [5]), was grown and protoplasts were prepared as in [1,6].

The protoplasts were gently lysed by the polybase method [7]. The lysate (1.5 ml with 7.5×10^8 protoplasts) was centrifuged (8000 × *g*, 10 min, 0°C) in a conical centrifuge tube which allowed efficient separation of the supernatant (soluble fraction, containing mainly cytosol) from the sediment (crude organelle fraction, containing almost all the vacuoles besides other cell organelles as determined by measuring marker enzymes). For comparison, sediments of intact protoplasts were also prepared and assayed under the same conditions. The soluble fraction was concentrated ~10-times by ultrafiltration.

Activation of trehalase was routinely performed in a reaction mixture (0.1 ml) containing 50 mM Sörensen-P_i buffer (pH 6.2), 2% (w/v) Triton X-100, 1 mM phenylmethylsulfonylfluoride, 8 mM ATP Na^+, 50 μM adenosine 3',5'-monophosphate (cAMP), 8 mM $MgCl_2$, and the enzyme sample (≥20 mg protein/ml). After incubation at 30°C for 10 min, a solution (0.9 ml) of ice cold 50 mM Sörensen-P_i buffer (pH 7.0) with 0.2 mM EDTA was added. Thereafter, trehalase activity was assayed immediately in a mixture (0.2 ml) conaining 50 mM trehalose, 100 mM Sörensen-P_i buffer (pH 6.2), 2 mM $MgCl_2$, and enzyme sample. After incubation at 30°C for 1 h the reaction was stopped by heating in a bath of boiling water (3 min) and the glucose released from trehalose

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was determined with the GOD-test from Boehringer GmbH (Mannheim). Activities of marker enzymes were determined as in [6].

3. RESULTS

Preincubation of a crude preparation of lysed protoplasts in the presence of cAMP and ATP results in a 40-fold increase of trehalase activity (table 1). The activation, clearly depending on both ATP and cAMP, proceeds very rapidly; it is completed within ~5 min in accordance with results in [4].

Table 1

Change of trehalase activity in a yeast protoplast lysate upon preincubation with cAMP and ATP

Preincubated with		Trehalase activity	
cAMP (μM)	ATP (mM)	pkat/mg protein	relative values
0	0	37	1
5	4	1259	34
50	4	1315	36
500	4	1268	35
50	8	1453	40
50	0	222	6

By centrifugation of a protoplast lysate which was prepared by the gentle but very efficient method polybase-induced lysis [7], two forms of trehalase can easily be separated, namely an active form of trehalase (Ta) and an inactive, zymogen-form (Tz) which can be activated by incubation with cAMP and ATP: Tz is found in the supernatant and Ta in the sediment (table 2). Upon preincubation in presence of cAMP and ATP the trehalase activity in the supernatant is increased >40-times, whilst in the sediment the activity is hardly doubled. As a protein kinase is presumably involved in the activation [2] the possibility had to be considered that not enough protein kinase was present in the sediment to allow full activation. The sum of the Tz-activities in the supernatant and the sediment found after activation is not much less than the activity in the protoplast lysate (table 2). This already shows that not much loss of activity, if any, is caused by insufficient activation. Experiments with preparations differing in protein kinase activity were performed by mixing sediment with supernatant or protoplast lysate in different proportions. The recovery of trehalase activities was tested in these mixtures. The results show that the activity of the sediment fraction cannot be increased further by adding supernatant or protoplast lysate during activation. Protein kinase is therefore most probably not lacking in the sediment. On the contrary, it was observed that the

Table 2

Distribution of the active form and the zymogen form of trehalase (Ta and Tz, respectively) and of marker enzymes in the supernatant and the sediment (8000 × g, 10 min) of a yeast protoplast lysate (Activities)

			Vacuolar markers		Cytosolic markers		Mitochondrial marker (MDH)
	Ta	Tz	α-MAN	RNase	G-6-P-DH	α-GLC	
Supernatant (U)	7	97	23	24	94	93	26
$U + S = 100\%$							
Sediment (S)	93	3	77	76	6	7	74
Protoplast lysate (P)							
(pkat/mg protein)	(40)	(1453)	(29)	(1.32)[a]	(3100)	(4)	(19200)
$(U + S)/P$ (%)	107	82	121	92	108	106	78

[a] Difference in absorbance at 260 nm; assay as in [6]

Abbreviations: α-MAN, α-mannosidase; G-6-P-DH, glucose-6-P-dehydrogenase; α-GLC, α-glucosidase; MDH, malate dehydrogenase

presence of the sediment-fraction has a slightly repressive effect on the total activities in the mixtures. This is interpreted to mean that trehalase is not very stable in the presence of large amounts of sediment-fraction. Possibly trehalase is attacked by the vacuolar proteinases much enriched in this fraction [6].

The distribution of subcellular components between supernatant and sediment was determined by the assessment of several marker enzymes. The activities of cytosolic enzymes (α-glucosidase, glucose-6-P dehydrogenase) are largely found in the supernatant together with Tz (table 2). This indicates that the protoplasts were efficiently lysed; intact protoplasts which survived the polybase-induced lysis would have carried cytosolic enzymes into the sediment. Hence Tz is most probably located in the cytosol. The possibility that it might be associated with light membranes not sedimentated under the conditions of the low-speed centrifugation applied, was excluded by a further centrifugation of the supernatant at $100000 \times g$ for 1 h; all Tz remained in the supernatant (not shown).

The activities of marker enzymes for vacuoles (α-mannosidase, ribonuclease, see [6]) were contained mostly in the sediment together with Ta (table 2). This result confirms the localization of Ta in vacuoles [1].

4. DISCUSSION

Assuming that the trehalase-zymogen (Tz) in the cytosol is indeed the precursor of the active trehalase (Ta) in the vacuoles, the obvious question arises whether cAMP-dependent phosphorylation of trehalase induces not only activation (transformation of Tz to Ta) but also initiates the transfer of the enzyme from the cytosol into the vacuoles.

If Ta is exclusively located in the vacuoles, as may be derived from the results, the substrate trehalose, which appears to be located in the cytosol [1], would have to be transported into the vacuoles for degradation. However, as Tz most probably is activated in the cytosol it is feasible that some Ta occurs also in this compartment, at least temporarily. Hence, trehalose may be degraded there. In this case, trehalose degradation could be initiated rapidly by the cAMP-dependent activation of trehalase. By the uptake of Ta into the vacuoles trehalose mobilization in the cytosol may then eventually be terminated. In this context it is interesting that after a phase of trehalase induction in yeast the trehalase activity always decreases again quite rapidly [1,3,4]. Possibly Ta is unstable in the vacuoles as these are the lysosomes of yeast [6].

A very similar sequence of events may occur during 'catabolite inactivation' of fructose-1,6-bis-Pase [8,9]. Addition of glucose to yeast cells grown on acetate or ethanol induces a rapid increase of [cAMP] and concomitant phosphorylation and extensive inactivation of fructose-1,6-bis-Pase. Thereafter, more slowly, the antigenic reactivity of fructose-1,6-bis-Pase disappears, indicating that the enzyme is proteolysed, possibly also in this case after having been transferred into the vacuoles [8].

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