Activation of yeast mannan synthetase by α factor pheromone

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The *Saccharomyces cerevisiae* mating pheromone α factor induces the activation of yeast mannan synthetase both in vivo and in vitro. In vivo the activating effect of the pheromone on the synthetase is specific for cells of the *a* mating type, while in vitro α factor is able to exert its action on the synthetase of either cell type (MAT *a*, MAT α and MAT *a*/MAT α).

Saccharomyces cerevisiae *Mannan synthetase* α *factor*

1. INTRODUCTION

The cell wall of *Saccharomyces cerevisiae* plays a central role in the process of sexual conjugation, in which haploid cells of opposite mating type fuse to form diploid zygotes. During this process the cell surface exhibits increased agglutinability in cell–cell contacts [1,2] and the cell wall undergoes structural and morphological alterations [3] previous to the formation of the mating pairs. These changes are elicited by the action of sex-specific pheromones secreted into the medium by each haploid cell type (*a* and α), acting reciprocally on cells of the opposite mating type (review [4]).

The mating pheromone α factor, secreted by MAT α cells, arrests growth of MAT *a* cells in the G1 phase of the cell division cycle [5] and induces changes in the morphology (pear-like shape) [6], agglutination properties [7–10] and in the structure [11] of their cell walls. It has been reported [11] that the morphological changes induced in MAT *a* cells by α factor can be blocked by cycloheximide and by inhibitors of cell wall biosynthesis and that α factor-treated MAT *a* cells contain more chitin [2], more glucan and less mannan [1] than untreated cells. These data suggest that α factor may act, directly or indirectly, on the catalytic reactions responsible for the biosynthesis of cell wall polymers. We show here that α factor has the ability to activate the glycosylation reaction(s) involved in the synthesis of yeast mannoproteins.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

Synthetic α factor was obtained from Peninsula Laboratories. GDP-mannose, α-melanocyte stimulating hormone, oxytocin, glucagon and insulin were from Sigma. GDP-[U-^{14}C]mannose (203 mCi/mmol) was purchased from The Radiochemical Centre, Amersham. All other reagents were of analytical grade.

2.2. *Yeast strains and culture conditions*

The standard wild-type haploid strains of *S. cerevisiae* X 2180-1A (MAT *a*) and X 2180-1B (MAT α), and their parental diploid strain X 2180 (MAT *a*/MAT α) were obtained from the Yeast Genetics Stock Center, Donner Laboratory, Berkeley, CA.

Cells were grown to early exponential phase in a defined medium containing 0.67% yeast nitrogen base, without amino acids (Difco) and 2% glucose, at 30°C, on a rotary shaker at 200 rpm.

2.3. *Enzyme preparation*

Washed cells were resuspended in 300 mM Tris–maleate (pH 7.0), 5 mM $MnCl_2$ and 1 mM mercaptoethanol (this buffer was used throughout the work) at a concentration of 1 g wet weight of cells per ml and broken in a Braun homogenizer

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for 2–3 min, under dry ice. The homogenate was spun down at 1500 × *g* for 5 min to remove unbroken cells and cell walls, and the resulting supernatant fluid centrifuged at 48 000 × *g*, for 30 min. The final pellet was washed once and resuspended in buffer at a protein concentration of ~ 30 mg/ml. All operations were performed at 0–4°C. Protein was measured according to [13].

2.4. *Mannan synthetase assay*

Mannan synthetase was assayed by measuring incorporation of [^{14}C]mannose from GDP-[U-^{14}C]mannose into trichloroacetic acid precipitable material. Standard assay mixtures contained 0.05 μCi GDP-[U-^{14}C]mannose, 2 mM GDP-mannose, ~ 300 μg enzyme protein and buffer, at a final volume of 50 μl. Samples were incubated for 1 h, at 30°C, and the reaction was stopped by adding 1 ml of cold trichloroacetic acid. The precipitates obtained were collected on GF/C filters (Whatman), washed with 10% trichloroacetic acid and the radioactivity counted in a toluene-based scintillation liquid with a Beckman LS-8100 scintillation spectrometer.

When it was necessary to determine the radioactivity incorporated into dolichol-derived products, the reaction was stopped by adding 1 ml of chloroform/methanol (2:1, v/v). The lipids were extracted by continuous shaking at room temperature for 3 h. The insoluble material was removed by filtration and the filtrates were washed as in [14]. The chloroform phase was brought to dryness and the radioactivity counted as above.

3. RESULTS

3.1. *Activation of mannan synthetase by α factor*

Fig. 1 shows that when cell-free extracts of *S. cerevisiae* were incubated under standard conditions in the presence of α factor there was an increase in the amount of [^{14}C]mannose incorporated, from GDP-[^{14}C]mannose, into trichloroacetic acid precipitable material. The activation of the incorporation was not directly proportional to the amount of α factor added to the reaction mixture and, under the conditions of the experiment, final concentrations of α factor higher than 23.7 μM (2 μg/50 μl) were not able to induce an activation greater than about 280% over the control without α factor.

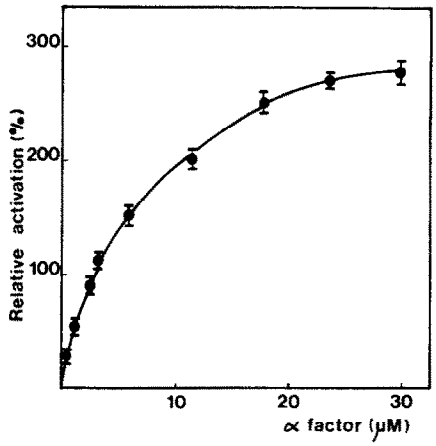

Fig. 1. Activation of mannan synthetase of cell-free extracts from *S. cerevisiae* X 2180-1A by α factor. Results are the mean of 4 separate experiments.

As shown in table 1 in both the absence and presence of α factor, less than 10% of the radioactivity incorporated into trichloroacetic acid precipitable material was found in compounds that could be extracted with chloroform/methanol, which presumably are dolichol-derived products [15]. Consequently, the pheromone seems to activate the incorporation of mannose into mannoprotein, which has been proved to constitute most of the radioactive material present in the insoluble fraction [16,17].

Table 1

Effect of α factor on the transfer of [^{14}C]mannose from GDP-[U-^{14}C]mannose to endogenous acceptors

Radioactivity incorporated into	In vitro[a]		In vivo[b]	
	Control	+α	Control	+α
Trichloroacetic acid precipitable material	13260	50350	5070	9100
Chloroform/methanol soluble material	1120	3390	nd[c]	nd[c]

[a]Final concentration of α factor was 23.7 μM. Values are the mean of 4 separate experiments and represent cpm/mg protein

[b]Values are the mean of 4 separate experiments and represent cpm/mg protein per 10^9 cells

[c]nd, not determined

α factor was also able to activate the mannan synthetase of cells treated in vivo with the pheromone. In a typical experiment, yeast cells from an early exponential phase culture were resuspended in fresh medium at a density of 5–8 × 10^6 cells/ml and supplemented with α factor (0.5 μg/ml). After 2 h of incubation at 30°C in an orbital shaker (200 rpm), when 100% of the cells had adopted the typical pear-like morphology induced by the pheromone, the cells were harvested and cell-free extracts prepared. Treated cells yielded cell-free extracts that incorporated about 80% more [^{14}C]mannose than a control extract obtained from untreated cells, expressing activity in terms of 'cellular specific activity' ([^{14}C]mannose incorporated/mg protein per cell) (table 1).

Yeast mannan synthetase is a multienzyme complex, probably constituted by a minimum of 10 different mannosyltransferases [18]. Assuming that this complex should behave as a single enzyme which catalyzes the transfer of mannose from the donor GDP-mannose to endogenous acceptors present in the cell-free extract, and because this reaction has been reported to follow a Michaelis-Menten kinetics [19], we calculated an apparent activation constant for the α factor of 3.1 μM, when mannan synthetase is assayed in the presence of the pheromone (not shown).

3.2. *Specificity of the activation*

The activating effect of α factor on mannan synthetae was specific for cells of the *a* mating type, when the treatment with the pheromone was carried out in vivo. Neither cell-free extracts obtained from treated MAT α cells nor those obtained from treated diploid cells exhibited a higher mannan synthetase activity, with respect to controls of untreated cells (not shown). However, as shown in fig. 2, α factor was able to activate in vitro not only the mannan synthetase present in cell-free extracts obtained from MAT *a* cells but also that from MAT α and diploid cells.

To check whether or not the action of α factor on mannan synthetase was specific we studied the effect of other low-M_r peptides on the activity of the synthetase, α-melanocyte stimulating hormone, oxytocin, glucagon or insulin were included in the standard reaction mixture, at final concentrations of 2.4 and 10 μg/ml, the first, and 20, 40 and 80 μg/ml, the other three. None of these peptides was able to increase the incorporation of [^{14}C]mannose over the control without any addition (not shown).

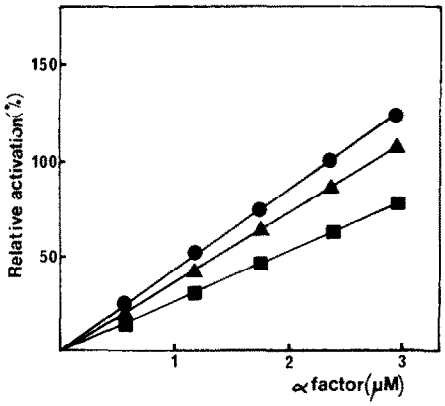

Fig. 2. Effect of α factor on the mannan synthetase activity of cell-free extracts obtained from MAT *a* (●), MAT α (■) and MAT *a*/MAT α (▲) yeast cells. Results are the mean of 4 separate experiments.

4. DISCUSSION

From our results it seems clear that α factor has the ability to activate the incorporation of mannose, from GDP-mannose, into trichloroacetic acid precipitable material, catalyzed by the so-called mannan synthetase. As has been already pointed out, this enzymatic activity may be mediated by at least 10 different enzymes [18] none of which have been purified nor whose conditions have been established for assaying them individually. Consequently, we do not know whether α factor acts on one, several or all of the enzymes involved in the synthesis of yeast mannoproteins.

Mannan synthetase is activated more efficiently by α factor in vitro than in vivo. This fact, together with the apparent inability of the pheromone to penetrate inside the cell [20], suggests that in vivo the action of α factor could be exerted only on the mannosyl transferases which are present in the plasma membrane [21–23]. Breakage of the cells could expose the intracellular enzymes of the mannan synthetase complex to the action of the pheromone. Likewise, reasons of accessibility could explain why, in vivo the α factor specifically activates the mannan synthetase of MAT *a* cells, while in vitro it is able to exert its activating effect on the synthetase of either cell type (MAT *a*, MAT

α and MAT a/MAT α). A similar lack of specificity in vitro has been reported for the inhibitory effect of α factor on the membrane-bound adenylate cyclase [24].

Regarding the biological significance of the effect of α factor on mannan synthetase, it is known that MAT a cells treated with α factor exhibit an altered immunochemical reactivity due to the synthesis of a specific antigen containing an acid-labile mannose determinant [25]. Fluorescent antibodies directed against α 1→3 mannosyl units bind preferentially to the tips of elongated, α factor-treated, MAT a cells [25], and a similar result has been obtained with fluorescent concanavalin A [26]. The tips of elongated cells are the critical sites of adhesion during conjugation [3], which is mediated by sex-specific agglutinating substances induced by the mating hormones [1,2, 7–10] some of which have been shown to be glycoproteins [27,28]. It is possible that α factor might act on the mannan synthetase by specifically activating the glycosylation reactions involved in the synthesis of those sex-specific glycoproteins which mediate the fusion of cells of opposite mating type previous to the formation of zygotes.

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