PURIFICATION, GLYCOSYLATION AND SECRETION OF A REPRESSIBLE ACID PHOSPHATASE OF Yarrowia lipolytica

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Abstract

A repressible acid phosphatase of Yarrowia lipolytica was purified and antibodies directed against its proteic portion were obtained (1). Immunoprecipitation experiments have made it clear that this enzyme is very heterogeneous. Such experiments have also led to the detection of a precursor of about 86 kDa in cells grown under conditions of derepression. This precursor corresponds to a partially glycosylated form of the enzyme. The heterogeneity shown by the enzyme is due to its oligosaccharidic component since a single sharp band of 58-60 kDa appears when the immunoprecipitates of the cellular extracts are treated with endo H. When the extracts from derepressed cells treated with tunicamycin were immunoprecipitated with the antibodies, two polypeptides of 54 and 52 kDa appeared. Preliminary experimental evidence supports the idea that these must be the mature non-glycosylated enzymatic protein and its precursor containing the signal sequence. Under normal conditions of derepression, cells secrete a glycosylated polypeptide into the culture medium, whereas in the presence of tunicamycin two polypeptides of 38 and 20 kDa, which are related immunologically to phosphatase, are detected in the culture medium.

Introduction

Acid phosphatases (EC 3.1.3.2) have been described in many yeasts (2). These enzymes are derepressed upon inorganic phosphate starvation, possibly to hydrolyze the organic phosphates, and hence provide orthophosphate for the cell. The purification and physicochemical characterization of several yeast acid phosphatases have been described (2) and intensive studies have addressed their synthesis (3), cellular localization (4), secretion and maturation (5), carbohydrate content (6) and regulation and genetic analysis (5). Also the PHO5 of Saccharomyces cerevisiae and PHO5-derived promotors have been used for regulated heterologous gene expression (7).
However, secretion in S.cerevisiae might be the limiting step in the production of foreign proteins (8) and thus many studies are now directed to finding other yeasts with higher secretion capacities. Within this context our interest was centered in another yeast Yarrowia lipolytica. In Y.lipolytica transformation systems have been described (9), the yeast is amenable to genetic and biochemical analysis (10,11) and has been used industrially (12); it also has potential as a host for the secretion of foreign proteins. The secretion and processing of one alkaline and three acid proteases and one RNAse of this yeast have been described (13-15). In view of this, in the present study we were prompted to use the acid phosphatase of Y.lipolytica in order to gain understanding of the glycosylation and secretion process.

Materials and Methods

Organisms and growth, conditions: Yarrowia lipolytica strain CX-39-74C Mat A ura 1, was kindly supplied by Dr. J.Bassel. Donner Laboratory. University of California. Berkeley.
Enzyme activity, Endo ß-N-acetylglucosaminidase H treatment, electrophoresis, electroblotting and immunological detection, amino acid analysis and antiserum preparation were performed as described previously (1). Preparation of labelled cell extracts, supernatant media and immunoprecipitation conditions were essentially as described in reference (14).

Results

An acid phosphatase from derepressed cells of the yeast form of Yarrowia lipolytica was purified by ammonium sulfate precipiation, chromatography on DEAE-Sephacel and gel filtration in Biogel A 5-M.

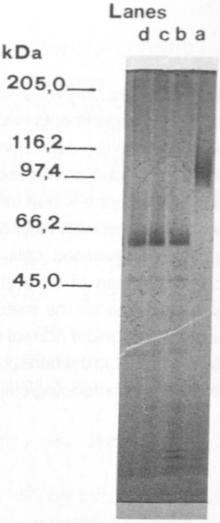


Fig. 1. SDS-acrylamide slab gel electrophoresis of acid phosphatase after treatment with Endo H. Lane a, control; Lanes b, c and d after 2, 12, 24 h of incubation with 1 mU of Endo H/μg protein.

Table 1.

Amino acid composition of Yarrowia lipolytica acid phosphatase. Comparison with Saccharomyces cerevisiae (16).

Amino acid	Y.lipolytica moles/60,000 g	S.cerevisiae moles/60,000 g
Aspartic acid	69-72	66-67
Threonine	54	38
Serine	48-51	33-35
Glutamic acid	57	38-40
Proline	39	12
Glycine	51-54	31-32
Alanine	51	26-27
Cysteine	-	10
Valine	30-34	26
Methionine	3	6
Isoleucine	29	21
Leucine	30	34
Tyrosine	15	30-31
Phenylalanine	21	25-26
Lysine	24	23
Histidine	9	9-10
Arginine	12	13
Tryptophan	-	-

The enzyme is a glycoprotein with a sugar content of 60%. The purified enzyme shows size heterogeneity within a linear concentration gradient of polyacrylamide (4-20%) which indicates an apparent molecular weight in the range of 90,000-200,000 in the presence of sodium dodecyl sulfate. The heterogeneity of the enzyme is due to its carbohydrate content, as can be demonstrated by gel filtration and by treatment with endoglycosidase H, as shown Fig. 1. The carbohydrate-depleted protein has a molecular weight of 60,000, and its amino acid composition is shown in Table 1 (1,16). The effect of the inhibition of protein on acid phosphatase synthesis is shown in Fig. 2. Under derepression conditions the external enzyme activity increased uniformly. When trichodermin (Rodriguez et al, Exp.Mycol. in press) was added to the culture medium, the increase in the external enzyme ceased after 40-60 min. The effect of tunicamycin on the formation of active acid phosphatase is shown in Fig. 3. Acid phosphatase activity was completely suppressed.

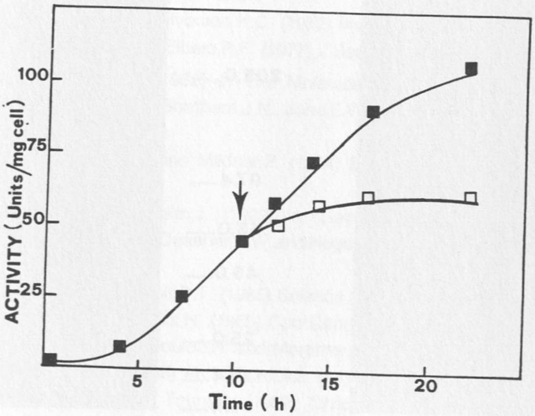


Fig. 2. Effect of trichodermin on acid phosphatase derepression.
Cells in high phosphate medium were transferred to low phosphate medium after 10 h of incubation (arrow). Trichodermin at 3,5 μg/ml was added. Samples were taken at the times indicated and the activity determined. (■), control culture; (□), trichodermin.

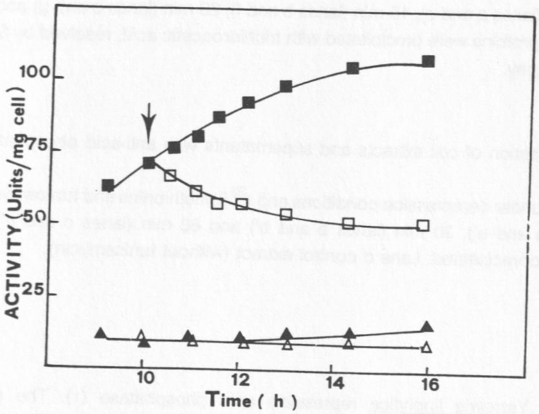
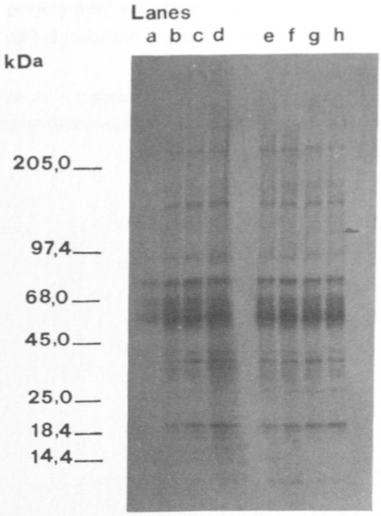


Fig. 3. Effect of tunicamycin on the formation of active acid phosphatase.
Cells in high phosphate medium were transferred to low phosphate medium. After 10 h of incubation (arrow) tunicamycin at 10 μg/ml was added. Samples were taken at the times indicated and the activity determined. (■), control cells; (□), tunicamycin treated cells; (▲), supernatant; (△), tunicamycin treated supernatant.

Labeling and immunoprecipitation conditions. Several polypeptides were obtained from the Y.lipolytica (^{35}S) methionine-labeled cell extracts under derepression conditions (Fig. 4). However, when tunicamycin was added under derepression conditions and cell extracts and supernatants were immunoprecipitated, only two polypeptides, one of them of about 86 kDa- which probably corresponds to a partially glycosylated form of the enzymes and another band of 58 kDa- appeared in the cell extracts and two polypeptides of 38 and 20 kDa appeared in the culture medium (Fig. 5).



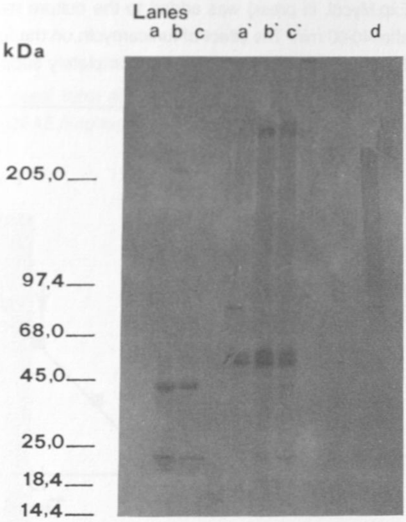


Fig. 4. Time course of secretion of (^{35}S)methionine-labeled polypeptides.
Cells suspended under derepression conditions were labeled after 10 h of incubation for 2 min. (lanes a, b, c and d) and 30 min (lanes e, f, g and h) and the label was chased with and excess of unlabeled l-methionine for 5 min (lanes a and e), 15 min (lanes b and f), 25 min (lanes c and g) and 40 min (lanes d and h). Extracellular proteins were precipitated with trichloroacetic acid, resolved by SDS-PAGE and visualized by fluorography.

Fig. 5. Immunoprecipitation of cell extracts and supernatants with anti-acid phosphatase antibodies: Effect of tunicamycin.
Cells were incubated under derepression conditions and (^{35}S)methionine and tunicamycin were added. After 10 min (lanes a and a'), 30 min (lanes b and b') and 60 min (lanes c and c') samples were collected and immunoprecipitated. Lane d control extract (without tunicamycin).

Discussion

We have purified a Yarrowia lipolytica repressible acid phosphatase (1). The enzyme is very heterogeneous due to its oligosaccharidic moiety. Treatment of the enzyme or of immunoprecipitates which Endo H produced a single sharp band of 58-60 kDa. In the presence of tunicamycin two polypeptides of 54 and 52 kDa appeared in cell extracts, indicating that differences in the processing of the enzyme occurs. However in the presence of tunicamycin the cell secreted two polypeptides of 38 and 20 kDa to the culture medium. Neither peptide appeared in cell extracts. The results indicate that in the presence of tunicamycin either the 58 kDa polypeptide is cleaved during its transport outside the cell or that in the medium, there exists a protease that specifically cleaves the peptide. Experiments to test this hypothesis are currently under way.

Acknowledgments

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