LOCALIZATION OF INVERTASE IN YEAST VACUOLES

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1. Introduction

The general characteristics, localization and secretion of yeast invertase (β-D-fructofuranoside fructohydrolase, EC. 3.2.1.26) have been studied in considerable detail. In derepressed cells most invertase is located outside the cytoplasmic membrane, and is lost from the cells upon protoplast formation. The internal enzyme is not affected by this process [1, 2].

Invertase isozymes have been found in *Saccharomyces* and they can be separated on Sephadex G-200 columns in to a heavy and a light form [3]. External invertase is all in the heavy form; the light invertase is contained within the protoplast. The heavy invertase is a glycoprotein containing 50% mannan and 3% glucosamine whereas the light enzyme contains no carbohydrate [4–6].

Some hydrolytic enzymes have been demonstrated in yeast vacuoles [7] and this prompted us to search for the intracellular localization of invertase.

In the present paper we report our studies on the localization of invertase inside the protoplasts. We have found that the internal invertase is localized in the vacuoles. Both heavy and light invertase are present in the vacuoles at similar concentrations.

2. Materials and methods

Two yeast strains were used, *Saccharomyces* strain 303-67 [8] generously supplied by Dr. P. Ottolenghi and a mutant of this, strain FH4C [9], kindly supplied by Dr. J.O. Lampen.

The cells were grown in Winge's medium with 3% glucose for 18 hr at 28°. They were subsequently reinoculated in a fresh medium containing 1% glucose and incubated for 3 hr. The cells were collected by centrifugation and washed three times in distilled water. The protoplasts were obtained and the invertase assayed as described previously [5]. The protein was measured according to Lowry et al. [10].

The lysis of protoplasts and liberation of vacuoles were achieved as described by Matile and Wiemken [7] by lowering the tonicity of the medium. Two volumes of a solution containing 0.025 M tris–citrate pH 6.5; 0.025% Triton–X-100 and 1 mM EDTA were added to one volume of a suspension containing 10^{10} protoplasts per ml in 0.6 M KCl.

3. Results

Table 1 shows the distribution of invertase and protein during isolation of vacuoles. As was already known, most invertase is lost in the supernatant during the preparation of protoplasts. Only a small fraction of the total cell protein is lost in this process and consequently the specific activity of invertase of protoplasts is lower than that of the original yeast cells. It is important to note this, because we are concerned mainly with the intracellular localization of invertase.

The distribution of invertase among fractions A, B and C after the release of vacuoles from protoplasts and subsequent centrifugation (table 1) clearly shows that the enzyme accumulates in fraction A, both with the preparation from *Saccharomyces* strain 303-67 and from the mutant FH4C. The specific activity of this fraction is higher than those of fractions B and C and intact protoplasts. In this scheme of fractionation the vacuoles are concentrated in frac-

Table 1
Distribution of invertase activity during the isolation of vacuoles from *Saccharomyces* strains 303-67 and FH4C.

Fraction	Activity (units/ml)		Protein (mg/ml)		Specific activity units enz./mg prot.	
	303-67	FH4C	303-67	FH4C	303-67	FH4C
Cells	0.78	72.5	6.24	10.5	0.12	6.9
Protoplasts	0.2	4.7	5.8	9.4	0.03	0.5
Fraction A	0.15	2.3	0.5	1.2	0.3	1.9
Fraction B	0.06	0.5	1.25	3.5	0.04	0.14
Fraction C	0.01	0.6	2.3	4.5	0.01	0.13

The cells were grown and the protoplasts were obtained and lysed as indicated under Materials and methods. Ficoll (Pharmacia Upsala) was added to the lysed protoplasts to a concentration of 8% (w/v); fraction C. The suspensions were overlayered with two layers of Ficoll 7.8% (fraction B) and 7.4% (fraction A). Most vacuoles were found in fraction A after centrifugation for 10 min at 3,000 *g*.

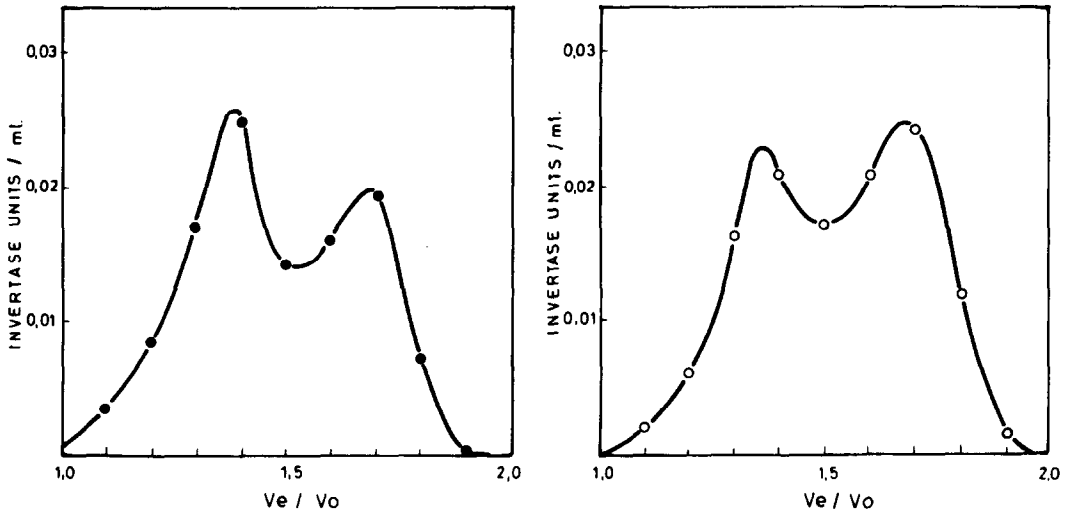

Fig. 1. Gel filtration in Sephadex G-200 of extracts of vacuoles. A homogenate of fraction A from table 1 containing 0.5 units of invertase was applied to a column of Sephadex G-200 (2 × 49 cm) equilibrated with 0.05 M tris-HCl buffer pH 7.5. Ve = elution volume; Vo = void volume of the column as estimated with Blue Dextran (Pharmacia) and detected by its absorption a 600 nm. Invertase from fraction A, ●—● strain FH4C; ○—○ strain 303-67.

tion A [7]. In our preparation, fraction A contained most of the vacuoles as could be shown in the phase microscope. Fraction B contained some vacuoles and fraction C was devoid of vacuoles but contained abundant cellular debris.

The elution pattern of vacuolar invertase from Sephadex G-200 is shown in fig. 1. Fraction A was homogenized in a Potter–Elvehjem, centrifuged at 15,000 *g* for 10 min, and an aliquot of the supernatant applied to the column. The results show that both heavy and light invertases are present in the vacuolar fraction. The amount of both isozymes present in the preparation was similar for both yeast strains.

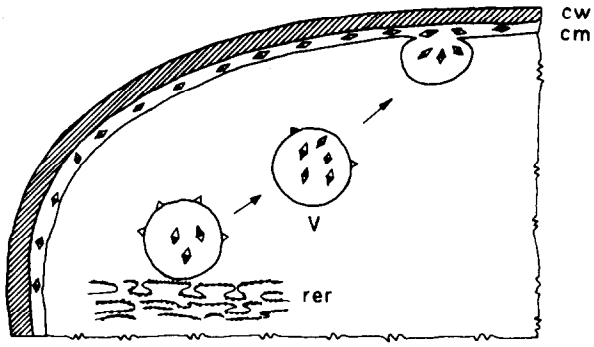

Fig. 2. Proposed scheme for the formation and secretion of invertase in yeast. RER = rough endoplasmic reticulum; V = vacuole; CM = cytoplasmic membrane; CW = cell wall; Δ light invertase (no carbohydrate); ♦ heavy invertase (50% mannan and 3% glucosamine).

4. Discussion

Most studies concerned with invertase have been focussed at the exoxellular enzyme, because this fraction usually represents more than 90% of the total invertase. The finding of isozymes of invertase [3] and the suggested biosynthetic relationship between the light and heavy enzymes [3, 6] prompted further studies in the biosynthesis and secretion of invertases.

Our data show that the invertase present in the protoplasts is found in the vacuolar fraction A. From the results of Matile and Wiemken [7] and from our observations, we can conclude that protoplasts invertase is located in the vacuoles. We obviously cannot rule out the possible significance of invertase present in fractions B and C.

It is interesting to note that both light and heavy invertase are present in the vacuoles. Until now it was not possible to state if the small amount of the large invertase remaining in the protoplasts was actually found inside the protoplast or could be due to incomplete removal of the cell wall [3, 5]. Our results clearly demonstrate that the heavy invertase is also present inside the protoplast.

A scheme of the formation and secretion of yeast invertase is shown in fig. 2. According to this working hypothesis the light invertase is probably synthesized in the rough endoplasmic reticulum or by ribosomes attached to the vacuolar surface. The addition of carbohydrate and the transformation of the light enzyme into heavy invertase could take place at the level of the vacuolar membrane. Reversed pinocitosis would be the final stage for the liberation of the heavy invertase into the extracellular space.

This hypothesis agrees with those postulated in animal cells for the secretion of amylase [11] and the need of attachment of carbohydrate as a passport for secretion [12, 13]. Recent work in yeasts gives support to the theory according to which vacuoles may be regarded as differentiation of the endoplasmic reticulum [14].

It would be interesting to test if the vacuoles we have isolated are similar to those obtained by other workers [15, 16] and to verify if they contain identical enzymatic complement as those described by Matile and Wiemken [7]. Further work is needed to elucidate the relative localization of light and heavy invertase in the vacuoles and the precise biosynthetic pathway.

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