LIGHT AND INTERMEDIATE MOLECULAR FORMS OF YEAST INVERTASE AS PRECURSORS OF THE HEAVY ENZYME

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Received 19 February 1980

1. Introduction

Yeast invertase (or B-D-fructofuranoside fructohydrolase, EC 3.2.1.26) has been found to exist in several molecular forms which differ not only in MW but also in cell localization [1]. The heavy enzyme is a glycoprotein containing 50% mannan and 3% glucosamine [2]. The light one contains no carbohydrate [3,4], and the intermediate molecular forms might represent different degrees of glycosylation of the enzymatic protein [5].

The cellular content of the different invertase molecular forms and their distribution in yeasts have been shown to be dependent on the glucose concentration of the culture medium, which is the main factor in controlling the synthesis of the enzyme [6–9]. In repressed cells the concentrations of the heavy and light forms of invertase are similar and most of the enzyme is intracellular. On the other hand, the heavy invertase represents 95% of the total enzyme in derepressed cells and this molecular form is located mainly outside the cytoplasmic membrane [10].

In the present paper, we report our studies on the relationship between the metabolic state of the yeast cells and the distribution of the different molecular forms of invertase in the yeast strain *Saccharomyces 303-67*. We introduce the intermediate molecular forms in the preexisting scheme [11], giving them a role as intermediates in the process of secretion of the heavy enzyme and as precursors of the exocellular invertase.

2. Materials and methods

2.1. *Materials*

Glucose oxidase (EC 1.1.3.4) type V, peroxidase (EC 1.11.1.7) type III and *o*-dianisidine were from Sigma Chemical Co.; Sephadex G-200 and Blue Dextran were from Pharmacia; the yeast extract was from Difco laboratories.

2.2. *Yeast strain*

The yeast strain used was *Saccharomyces 303-67*, kindly supplied by Dr P. Ottolenghi. The strain is diploid and homozygous for the SUC2 gene (R_2 of Winge and Roberts [12]). It synthesizes invertase in appreciable amounts only after the glucose has disappeared from the medium [9,13].

2.3. *Culture condition; preparation of cell-free extracts*

The yeasts were inoculated into flasks with 300 ml medium containing 1% yeast extract and 3% glucose and incubated in a rotatory shaker at 28°C. Harvesting after 16 h revealed that more than 1% glucose still remained in the culture supernatant. The cells were subsequently reinoculated in 300 ml fresh medium containing 1% yeast extract and 1% glucose and incubated for 6 h. Samples of 50 ml were removed at different times and their cells were collected and washed three times in distilled water by centrifugation at 4000 × *g* for 10 min.

Cell growth was measured as absorbance at 600 nm.

The cells were broken in a Braun MSK homogenizer with glass beads and the cell-free extracts were obtained by centrifugation at 20 000 × *g* for 20 min.

2.4. *Invertase assay*

The invertase was assayed in a two-step method as described previously [3]. One unit of invertase is defined as the amount of enzyme which hydrolizes 1 μmol of sucrose in 1 min at 30°C in 0.05 M sodium

acetate buffer, pH 5.0 containing 0.125 M sucrose.

Glucose concentration in the culture supernatants was assayed by this same method.

2.5. *Analytical gel filtration*

The quantitative analysis of invertase forms was carried out in Sephadex G-200 columns (2.5 X 90 cm) equilibrated and eluted at 4°C with 0.05 M Tris–HCl buffer, pH 7.5. The void volume (V_0) was determined from the elution volume of Blue Dextran, detected by its absorbance at 600 nm.

3. Results and discussion

Fig.1 shows a typical elution profile of the heavy and light invertase of two cell-free extracts obtained from repressed (A) and derepressed (B) cells, as detected by Sephadex G-200 filtration. These characteristic profiles were obtained when the glucose concentration in the culture medium was more than 1% (fig.1A) or when the glucose had disappeared from the culture medium some hours before harvesting the cells (fig.1B). In both cases the light invertase levels are similar, but important changes can be observed in the heavy enzyme, which could represent, in the case of derepressed cells, more than 90% of the total enzyme.

By following the distribution of the different invertase molecular forms in a culture of *Saccharomyces 303-67* during its growth, it has been possible to establish a correlation between the glucose levels in the medium and the proportion of heavy, light and intermediate molecular forms of invertase. It has also been possible to show how the MW of the intermediate molecular forms changes during the growth of the yeast. At different glucose concentrations the molecular weight varies from forms with a V_e/V_0 ratio near the light invertase to a V_e/V_0 ratio near the heavy enzyme.

Fig.2 summarizes the results obtained from this study. When the glucose concentration in the culture medium is high (figs.2A and 2B) it is possible to observe an intermediate molecular form of invertase with a V_e/V_0 ratio of 1.30. The change from a repression to a derepression state (fig.2C) entails an important increase of the heavy and intermediate forms of the enzyme, which is accompanied by a displacement of the V_e/V_0 ratio from 1.30 to 1.19 (figs.2D and 2E). It means that while the heavy invertase is being actively synthesized and secreted to

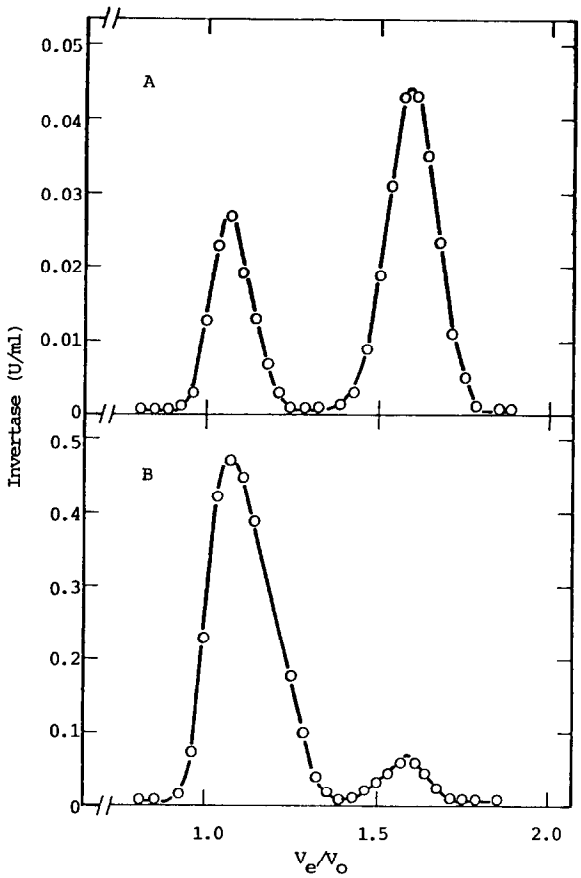

Fig.1. Gel filtration in Sephadex G-200 of cell-free extracts from *Saccharomyces 303-67*. In A the cells were repressed, that is, they were grown in a medium containing 1% yeast extract and 3% glucose, and more than 1% glucose was left in the culture supernatant at the time of harvesting. In B derepressed cells were used. They were grown in a medium containing 1% yeast extract and 1% glucose, which had disappeared at the time of harvesting. The extracts used contained 2.0 invertase units (A) and 20.0 (B), respectively.

the periplasmic space the intermediate molecular forms are also actively synthesized, accumulating inside the protoplasts [5] and being transformed to heavy invertase (figs.2E and 1B).

From these experiments we can conclude that the levels of the intermediate molecular forms of invertase depend on the metabolic state of the yeast, and that these forms change their MW from the light invertase (V_e/V_0 ratio of 1.65) to the heavy one (V_e/V_0 ratio of 1.07).

As shown in table 1, the amount of the light invertase per cell is not affected by the changes in the glucose concentration of the growth medium. This is not the case with the heavy and intermediate molec-

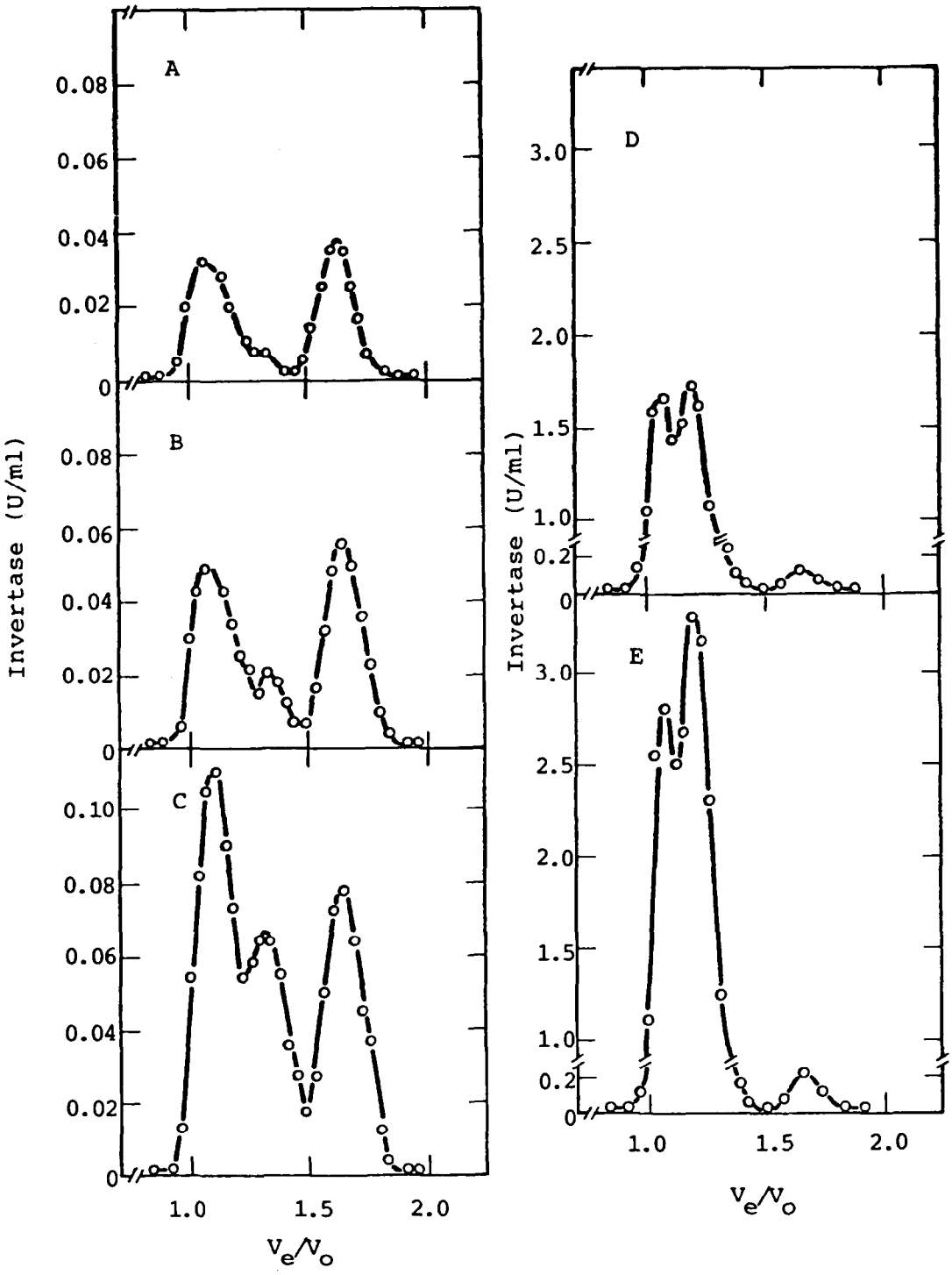

Fig.2. Analytical gel chromatography of invertase on Sephadex G-200. Cells of *Saccharomyces 303-67* were grown and the cell-free extracts were obtained under conditions described in Section 2. 8 ml samples of cell-free extracts were applied to a column of Sephadex G-200 (2.5 × 90 cm) equilibrated with 0.05 M Tris–HCl buffer pH 7.5. Cell-free extracts contained 3.3 units of invertase at 30 min of incubation (A); 4.6 at 60 min (B); 7.1 at 90 min (C); 75.0 at 315 min (D) and 127.0 at 345 min (E).

Table 1
Distribution of invertase molecular forms during a culture of *Saccharomyces 303-67*

Time (min)	Glucose (mM)	Molecular forms of invertase					
		Heavy		Intermediates		Light	
		U/10^8 cells	%	U/10^8 cells	%	U/10^8 cells	%
30	42	0.020	49	–	–	0.021	51
60	25	0.029	49	0.005	9	0.024	42
90	4	0.042	46	0.022	24	0.027	30
315	0	0.320	45	0.370	52	0.022	3
345	0	0.290	39	0.430	58	0.025	3

The yeast cells were grown as described in Section 2. At the times indicated, samples (50 ml) were removed and glucose concentration was determined in the culture supernatant. The cells were broken in a Braun MSK homogenizer and the cell-free extracts were applied to a Sephadex G-200 column, as described in the legend of fig.2. All values for activity are expressed as invertase units per 10^8 cells. Intermediate molecular forms correspond to the peaks of invertase activity eluting with a V_e/V_0 ratio between 1.07 (V_e/V_0 ratio of heavy enzyme) and 1.65 (V_e/V_0 ratio of light enzyme). The percentage of each form was calculated on the basis of total invertase activity (sum of heavy, intermediate and light invertase activities)

ular forms of the enzyme, which undergo important changes as the glucose concentration in the culture medium decreases. Expressing the data shown in table 1 as percentage of each form calculated on the basis of total invertase activity, it can be deduced that the intermediate molecular forms of the enzyme can represent up to 58% of the total invertase per cell in certain growth conditions.

A great number of attempts have been carried out in order to elucidate the biosynthetic relationship between the invertase isoenzymes [5,11,14–17]. It has been hypothesized that the light enzyme could be either a precursor [5,11] or a degradative product [14] of the heavy enzyme. We believe that the results of this study lend support to the idea that the light and intermediate molecular forms are precursors of the heavy invertase.

Acknowledgements

C. F. Iglesias was a recipient of a fellowship from the Ministerio de Universidades e Investigación. This work was supported in part by a grant from the Comisión Asesora de Investigación Científica y Técnica.

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