SECRETION OF INTERMEDIATE MOLECULAR FORMS OF INVERTASE BY *SACCHAROMYCES CARLSBERGENSIS* G-517 TREATED WITH 2-DEOXY-D-GLUCOSE

F. PARRA, P. HERRERO, F. MORENO and S. GASCON

Departamento Interfacultativo de Bioquimica, Universidad de Oviedo, Oviedo, Spain

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1. Introduction

Yeast invertase exists in several forms of different size and cellular localization: a heavy form located outside the plasma membrane [1,2] containing 50% mannan and 3% glucosamine [1], a light internal invertase which contains no glycan [3] and a continuous spectrum of soluble intracellular intermediate forms [4] or plasma membrane-associated forms [5,6] which would represent the sequential addition of mannose to the protein during the secretion process [4].

There is a continuing controversy over the role of the internal invertases in synthesis and secretion. The low molecular weight, unglycosylated, internal form of invertase may play no active role in the secretory process, but represent a metabolic dead-end [7]. However, the small form of invertase may be the precursor of the high molecular weight, glycosylated, secreted form [3,8], formed by processes involving the vacuole [9] which share some features of the mechanisms operating in higher eukaryotes.

Invertase biosynthesis in yeast is subjected to repression by hexose concentration in the culture medium [10]. The effects of the medium constituents on invertase formation have generally been related to carbon catabolite repression. In [11] we found that addition of 2-deoxy-D-glucose to cells fermenting galactose increased the synthesis of the enzyme, while in the presence of glucose this effect was not observed. We suggested that these findings were related to the catabolite repression [11].

Abbreviations. dGlc, 2-deoxy-D-glucose

Enzymes. invertase, β-D-fructofuranoside fructohydrolase (EC 3.2.1.26)

Here we report studies on the nature of the superproduced enzyme by cells treated with dGlc and the relationship between our results and the above secretion hypothesis.

2. Materials and methods

2.1. *Chemicals*

D-Galactose and 2-dGlc were from Sigma, Sephadex G-200 and blue dextran from Pharmacia and aquacide II from Calbiochem. The yeast extract and yeast nitrogen base were purchased from Difco L Labs. Other reagents used were of analytical grade whenever possible.

2.2. *Organisms and culture conditions*

The yeast strain used in this study was *Saccharomyces carlsbergensis* G-517 [12]. Cells were grown overnight at 28°C in flasks with 100 ml 1% yeast extract and 1% galactose. The cells were harvested by centrifugation at 4000 × *g* for 10 min and washed twice with distilled water. These cells were used to inoculate a fresh medium containing 1% yeast extract or 0.67% yeast nitrogen base according to the experiment and 10 mM galactose.

2.3. *Preparation of cell-free extracts*

Cells were harvested by centrifugation at 4000 × *g* for 10 min washed and then resuspended in 0.05 M Tris–HCl buffer (pH 7.5). The yeasts were broken in a Braun MSK homogenizer with glass beads and the cell-free extract was obtained by centrifugation at 48 000 × *g* for 30 min.

2.4. *Analytical gel filtration*

The gel filtration was performed in a Sephadex G-200 column (2.5 X 95 cm) equilibrated and eluted at 4°C with 0.05 M Tris–HCl buffer (pH 7.5) using reverse flow. The void volume (V_0) was determined from the elution volume of blue dextran, detected by its A_{600}.

The culture supernatant was concentrated before gel filtration by dialysis at 4°C against sodium salts of carboxymethyl-cellulose (aquacide II).

2.5. *Invertase assay*

Invertase was assayed according to [13] by measuring the liberated glucose from sucrose. One unit of invertase is defined as the amount of the enzyme which hydrolyzes 1 μmol sucrose in 1 min at 30°C in 0.05 M sodium acetate buffer (pH 5.0) containing 0.125 M sucrose.

3. Results and discussion

The data obtained when we assayed invertase activity in whole cells (external invertase)(fig. 1) showed that addition of 0.3 mM dGlc to cells fermenting galactose inhibited the synthesis of the enzyme for a certain time, after which invertase was actively synthesized reaching levels higher than the control culture without additions (invertase activity ratio of the 8 h treated culture:untreated = 1.86). In a [11] we discussed these findings postulating an interference with an enzyme in the galactozymase pathway, thus originating a lower catabolite repression and the above super-production of invertase.

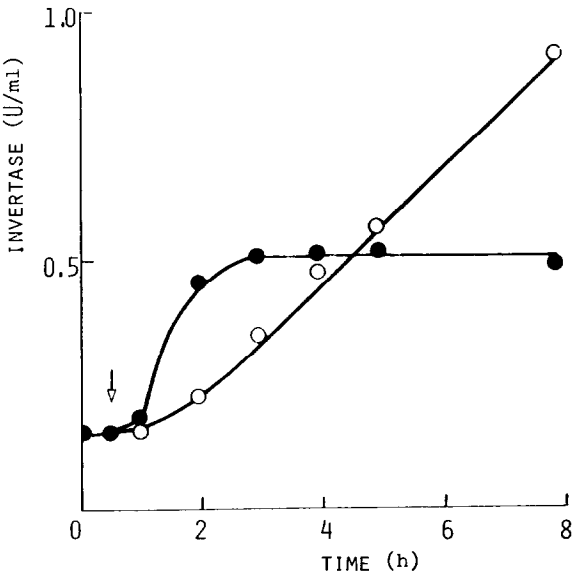

Fig.1. Effect of dGlc on the synthesis of the external invertase. Cells were grown in 1% yeast extract and 10 mM galactose. At the point shown by the arrow the culture was divided into 2 aliquots. Control without additions (•); + 50 μg dGlc/ml (○).

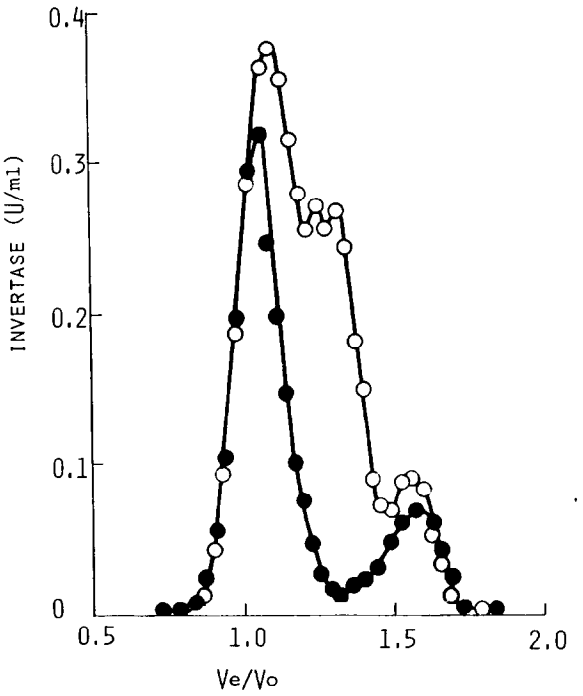

Fig.2. Gel filtration in Sephadex G-200 of cell-free extracts from *S. carlsbergensis* G-517. Cells from the culture represented in fig. 1 were harvested after 8 h incubation, and the cell-free extracts were obtained under the conditions in section 2. Untreated cell-free extract (•); + dGlc (○).

We were interested in clarifying if all the super-produced invertase was the heavy form of the enzyme or if there were other molecular forms of invertase outside the plasma membrane.

Equal samples of both cultures at 8 h incubation were taken up, cell-free extracts were obtained as in [11] and analyzed through a Sephadex G-200 column. The elution profiles shown in fig. 2 indicated that the levels of the heavy form of the enzyme ($V_eV_o = 1.06$) (the only extra-cellular form yet described), in both extracts were not quite different enough to explain the distinct invertase levels detected in whole cells. To explain these differences, it is necessary to assume that the intermediate molecular forms of the enzyme must

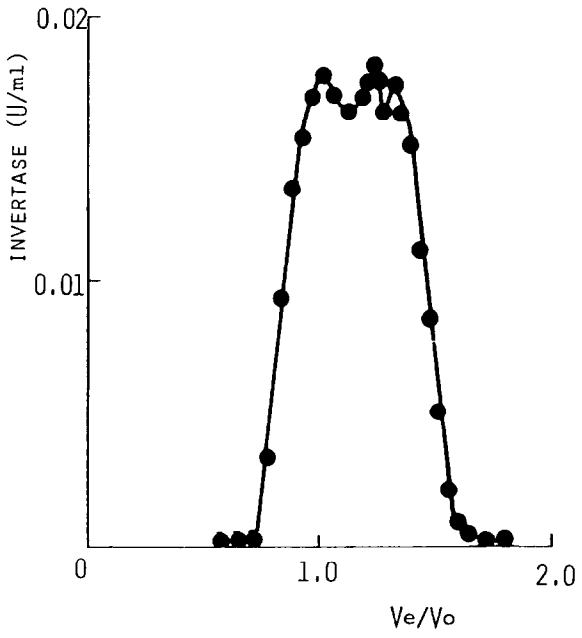

Fig.3. Gel filtration of a culture supernatent in Sephadex G-200. Cells were grown in yeast nitrogen base 0.67% and galactose 10 mM. After 30 min incubation at 28°C, 50 μg/ml of dGlc were added. After 7 h cells were harvested and the culture supernatant was concentrated 6.5 times by dialysis at 4°C against aquacide II.

be outside the plasma membrane. Direct evidence for this hypothesis was obtained when we analyzed in Sephadex G-200 a concentrated supernatant of a treated culture. In this case cells were grown in yeast nitrogen base medium to avoid the interference of the yeast extract in the concentration process. As we can see in fig. 3 the heavy form of invertase (V_eV_o = 1.06) was accompanied by others with V_eV_o ratios between the heavy and the light forms of the enzyme.

The biological significance of the carbohydrate moiety of glycoproteins has been less than obvious. Several functions have been suggested, such as transport of macromolecules or stabilization of the protein moiety structure [14]. Attachment of carbohydrate may be an essential element in the process of glycoprotein secretion. However, in myeloma cells, under conditions of dGlc inhibition, the absence of the carbohydrate retarded but did not prevent the export from the tumor cell of the non-glycolysated K-46 inmunoglobulin light chain [15]. Our results indicate that the intermediate forms of invertase (hypothetically less glycosylated) were secreted by cells, showing that the complete glycosylation of the enzyme was not essential for its release from the cell. An alternative explanation could be that under conditions of dGlc inhibition, the selective properties of the plasma membrane would be altered, permitting the transit through it of the less glycosylated forms.

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