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EXPERIMENTAL ARTICLES

Mutual Influence of Invertase and Acid Phosphatase of the Yeast *Saccharomyces cerevisiae* on Their Secretion into the Cultivation Medium

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Abstract—The hypothesis that various extracellular enzymes produced by the yeast *Saccharomyces cerevisiae* exert a mutual influence on their secretion into the cultivation medium was tested experimentally. The statistically processed results indicate that extracellular invertase affects the secretion of acid phosphatase, and acid phosphatase affects the secretion of invertase. In addition, the secretion of each of these enzymes was shown to be subject to autoregulation.

Key words: yeast, acid phosphatase, invertase, regulation of secretion.

The biosynthesis and transport of extracellular eukaryotic proteins is controlled through various mechanisms [1]. Feedback control of the synthesis of secreted enzymes was first described by Yurkevich for the α-amylase of *Aspergillus crysae* [2]. When added to the medium at a certain concentration, this enzyme caused the repression of the synthesis of the corresponding polypeptide chain. Syntheses of proteinases [3] and phosphohydrolases of *Penicillium brevicompactum* [4] and of the invertase of *Saccharomyces carlsbergensis* [5] were also shown to be regulated by their content in the medium. Repression of the synthesis is switched on when the enzyme interacts with the surface of the enzyme-producing cells. It was demonstrated that protoplasts of *Aspergillus* and *Saccharomyces* synthesize and secrete α-amylase and invertase, respectively, regardless of the enzyme content in the medium [6, 7]. The fact that the deglycosylated form of invertase cannot influence its own synthesis (although it retains its activity) suggests that the structure of the extracellular enzyme is important for autoregulation [8]. We previously studied how the expression of the genes encoding the extracellular enzymes α-amylase and invertase influence the secretion of the acid phosphatase [9]. It was demonstrated that acid phosphatase secretion was independent of the α-amylase gene expression but did depend on the expression of the invertase-encoding gene.

In this work, we test the hypothesis that the secretion of two extracellular glycoproteins, produced by the yeast *Saccharomyces cerevisiae*, the acid phosphatase (E.C. 3.1.3.2), and the invertase (E.C. 3.2.1.26), is mutually regulated by the concentrations of these two enzymes in the medium.

MATERIALS AND METHODS

This study used the yeast strain *Saccharomyces cerevisiae* S-288C obtained from P. Venkov at the Institute of Molecular Biology of the Bulgarian Academy of Sciences and strain S59-1-98-1G-P188, carrying the mutant gene of the constitutive acid phosphatase (this strain was obtained from M.N. Smirnov, Petergof Genetic Collection). Yeast cells were grown in liquid medium containing 2% peptone, 2% glucose, and 0.02 M succinate buffer, pH 5.0. In several experiments, the carbon source was replaced with another one and the phosphate concentration was altered in the medium as indicated in the text. Acid phosphatase activity was determined from the rate of hydrolysis of 2-nitrophenyl phosphate [10], and invertase activity was determined from the rate of the formation of reducing sugars during sucrose hydrolysis, measured using the dinitrosalicylic reagent [11]. The amount of enzyme hydrolyzing 1 μmol substrate in 1 min at 30°C was defined as 1 unit (E) of enzyme activity. Protein was assayed by Bradford's method [12]. Phosphorus was determined by Panusz's method [13]. Cell growth was estimated on a spectrophotometer at 500 nm. A single procedure was used to isolate acid phosphatase and invertase from the yeast culture medium. It included protein precipitation with cold 60% acetone followed by chromatography on

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a column with Ultrogel AcA 44 (LKB, Sweden). The extracellular invertase was isolated from the culture medium of the yeast strain S59-1-981G-P188. The activity of the preparation was 24 E/mg of protein. Extracellular repressible acid phosphatase was isolated from the culture medium of strain S288C. The activity of this preparation was 3.4 E/mg of protein. Lyophilized preparations of acid phosphatase and invertase were stored at –20°C and used to determine the effect of each enzyme on the secretion of the other one. The results were processed statistically according to Markov and Lisenkov [14].

RESULTS AND DISCUSSION

To evaluate the influence of extracellular yeast enzymes on their own secretion into the growth medium and on the secretion of other enzymes with similar structure, we used invertase and acid phosphatase as examples. Previously we established that, under certain conditions, the yeast *S. cerevisiae* produces acid phosphatase in amounts sufficient to be detected in the medium [15]. The amount of the acid phosphatase secreted depended not only on the inorganic phosphate concentration but also on the nature and content of the carbon and nitrogen sources in the medium [15]. An increase in glucose concentration in the medium to 6% led to the stimulation of acid phosphatase secretion, which was accompanied by a drop in the invertase content almost to zero. When glucose was replaced with glycerol, secretion of acid phosphatase decreased considerably, whereas invertase secretion increased. Thus, cultivation conditions favorable for one enzyme were unfavorable for the other. In further experiments, we used the cultivation conditions required for the secretion of a studied enzyme.

Note that both invertase and acid phosphatase are relatively stable enzymes and that their activity in the medium remained unchanged during the experiments (from 6 to 12 h). To study the effect of an enzyme added to the medium on the secretion of this or another enzyme, we used a standard technique: yeast cells grown to the early stationary phase and washed with sterile medium were transferred into tubes and incubated in appropriate media with various enzyme additions. Each of the experiments was run 5–10 times. The scatter of data is shown in the figures. In the experiments performed to study autoregulation, the concentration of the added enzyme was subtracted from that determined in the medium upon completion of the experiment. The results obtained were processed statistically using regression analysis and orthogonal polynomials. The level of enzymatic activity determined under the conditions used was characteristic of the early stationary-phase yeast cells and will be hereafter referred to as physiologically normal.

Strain S59-1-98-1G-P188, carrying mutant gene encoding constitutive acid phosphatase, was used to study the effect of invertase on its own secretion; invertase was added to the medium at a concentration corresponding to its physiologically normal level. Cells were grown in medium containing 6 mM orthophosphate and 0.5% glycerol as the carbon source. Under these cultivation conditions, the synthesis of repressible acid phosphatase does not occur either.

Regression analysis of the results obtained showed that invertase added in the medium to the final concentration of 1 E/ml stimulated the secretion of the same enzyme; the values obtained were statistically significant. At this enzyme concentration, the experimental points deviated from a straight line (Fig. 1) within the limits of experimental error. Higher invertase concentration led to a drop in the enzyme secretion. These data confirm Yurkevich's hypothesis suggesting that invertase secretion in yeasts is subject to autoregulation.

Figure 2 demonstrates the effect of extracellular invertase on the secretion of the repressible acid phosphatase. These experiments differed from those described above in that cells of strain S59-1-98-1G-P188 were transferred into phosphate-free medium after they achieved the early stationary growth phase. Under these conditions, derepression of the acid phosphatase synthesis occurred; if invertase was added to the medium at a concentration of 0.45–1.1 *E*/ml, the activity of extracellular acid phosphatase decreased to the same level. With a further increase in the content of extracellular invertase, the acid phosphatase activity in the culture liquid again increased.

Figure 3 illustrates the dependence of the secretion of repressible acid phosphatase on the amount of invertase added to the medium. Cells of *S. cerevisiae* S-288C were grown in a phosphate-free medium containing 0.5% glucose as the carbon source. As seen from the figure, acid phosphatase accumulation depended on the amount of invertase added to the medium (with a probability of more than 99%). This was a nonlinear dependence described by a third-order parabola. At an invertase concentration of 3.2–3.4 *E*/ml, the content of extracellular acid phosphatase increased, but a further increase in the invertase concentration in the medium led to a decrease in the extracellular acid phosphatase activity.

To elucidate the dependence of invertase secretion on the content of repressible acid phosphatase in the medium, cells of *S. cerevisiae* S-288C were cultivated in a medium containing 0.5% glycerol as the carbon source (Fig. 4). When the acid phosphatase concentration ranged from 0 to 0.9 *E*/ml, the extracellular invertase activity increased linearly. The ratio of the inadequacy dispersion to the reproducibility dispersion was 2.4 and did not exceed the critical magnitude of Fisher's criterion even at a 5% significance level.

Analysis of the effect of extracellular acid phosphatase on its own secretion showed that the secretion was inhibited at the threshold amount of this enzyme in the medium of 30–40 *E*/ml, which by an order of magnitude

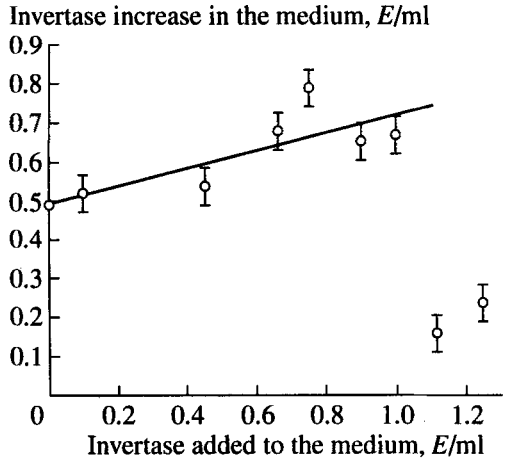

Fig. 1. Dependence of invertase secretion on the content of this enzyme in the medium.

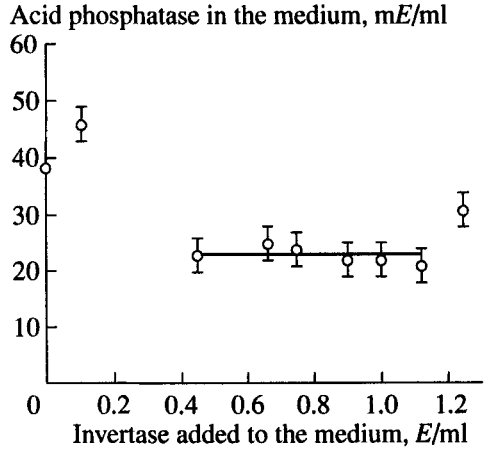

Fig. 2. Effect of invertase concentration on the secretion of repressible acid phosphatase.

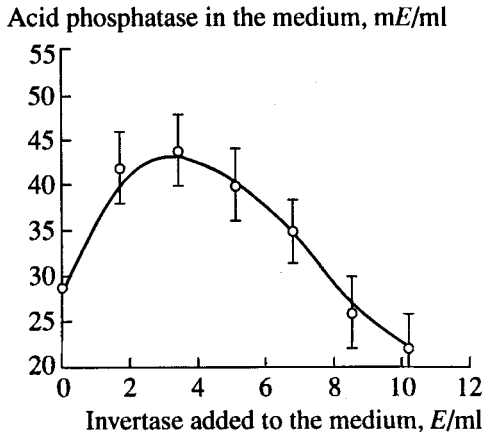

Fig. 3. Effect of invertase concentration considerably exceeding the normal level on the secretion of repressible acid phosphatase.

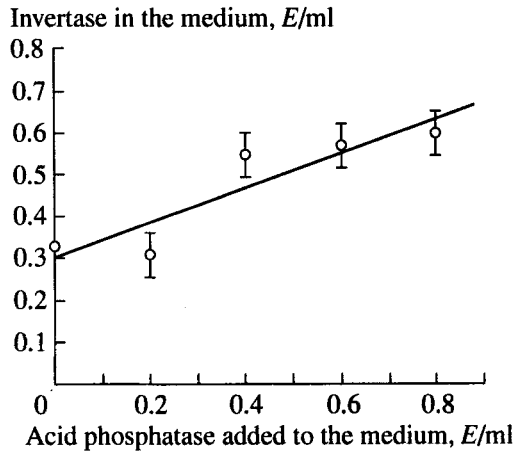

Fig. 4. Dependence of invertase secretion on the content of acid phosphatase in the medium.

exceeded its physiologically normal content in the medium under the experimental conditions used.

The presence of structural antigenic determinants shared by both extracellular enzymes studied would be a possible explanation of their mutual regulatory effect. To verify this suggestion, we obtained polyclonal rabbit antibodies against highly purified repressible acid phosphatase. These antibodies reacted neither with yeast invertase nor with yeast cell-wall mannan (Koch-Light Laboratories, United Kingdom), as judged from Ouchterlony's double diffusion method, whereas clear precipitation bands were obtained with preparations of both constitutive and repressible acid phosphatases. It is possible that the mutual effect of invertase and acid phosphatase on their secretion is related to the similarity between the subunit organization of these enzymes [17, 18] and the common vesicular mechanism responsible for the transport of these enzymes to the yeast cell wall [19].

Thus, our results suggest that regulation of the secretion of extracellular enzymes in yeasts involves, in addition to the well-known autoregulation, more complicated regulatory mechanisms, including the mutual influence of the enzymes secreted.

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