SC 11028

The acid phosphatase of yeast
Localization and secretion by protoplasts

Previous studies on yeast phosphatases indicated that the acid phosphatase (EC 3.1.3.2) was located externally on the surface of the yeast and that the alkaline phosphatases (EC 3.1.3.1) were internal[1-3]. SOUMALAINEN[3] found that the phosphatase activity could be increased by phosphate starvation. In contrast to the yeast phosphatases, the alkaline phosphatase of *Escherichia coli* is inducible and external, the acid phosphatase internal. The demonstration that the alkaline phosphatase of *E. coli* is liberated into the medium on conversion of the cells to protoplasts[4] is proof that the enzyme is located outside the cell membrane.

Phosphatase was assayed with nitrophenyl phosphate according to TORRIANI[5]. Acid phosphatase was measured at pH 4.0 and alkaline phosphatase at pH 8.9. A unit of activity is defined as the cleavage of 1 μmole of nitrophenyl phosphate per h at 25°. *Saccharomyces cerevisiae* LK2G12 was grown in minimal medium with 2% glucose[6], or phosphate was omitted and the medium buffered to pH 5.8 with citric acid. Protoplasts were prepared with snail enzyme as described previously[7].

Cells grown on $4 \cdot 10^{-2}$ M phosphate had less than 0.5 unit of acid phosphatase per 10^8 cells. Maximum phosphatase derepression was at $2.5 \cdot 10^{-4}$ M phosphate giving a level of 40 units of acid phosphatase per 10^8 cells. For the experiments described the cells were grown overnight in minimal medium, washed twice in phosphate-free medium, and then grown 4 h in the latter medium (absorbancy 0.15 → 0.4). Protoplasts were prepared from these log-phase cells. Complete formation of protoplasts could not be obtained if the cells were allowed to reach stationary phase (maximum phosphatase).

TABLE I

DISTRIBUTION OF PHOSPHATASE ACTIVITY

		Derepressed	*Repressed*
Acid phosphatase	Intact	16.3*	1.5
	Protoplast	0.3	0.1
	Lysed protoplast	1.3	0.4
Alkaline phosphatase	Protoplast	0.2	0.1
	Lysed protoplast	7.4	1.8

* Units/10^8 cells.

Table I shows the distribution of phosphatase activity in repressed and derepressed cells. The determination of acid phosphatase with intact cells or protoplasts measures only the surface phosphatase. This acid phosphatase was removed from the cells on preparation of protoplasts. (The small amount on the surface of the protoplasts can be accounted for by residual intact cells.) The acid phosphatase released on lysis of protoplasts is a measure of the internal acid phosphatase. The amount found was the same as the increase in acid phosphatase activity (about 1.0 unit/10^8 cells) on breaking the yeast with a French pressure cell.

Alkaline phosphatase is also an inducible enzyme (Table I). None was found

on the surface of the yeast and only negligible amounts on the surface of the protoplasts. Its activity was detected only after lysis of the protoplasts.

Recovery of the acid phosphatase released in preparing protoplasts was accomplished in a separate experiment. The incubation mixture contained $6 \cdot 10^9$ cells/ml, $5 \cdot 10^{-2}$ M mercaptoethanol, $3 \cdot 10^{-3}$ M EDTA, $3 \cdot 10^{-2}$ M $MgSO_4$, 0.5 M KCl, 0.1 M succinate buffer (pH 6.0) and 30% snail enzyme. Protoplasts were obtained after 40 min at 30°, centrifuged and washed with 0.5 M KCl 0.1 M succinate buffer. The cells had 6.9 units of acid phosphatase per 10^8 cells, the protoplasts 0.8 unit/10^8 cells. After correcting for the phosphatase activity of the snail enzyme, 80% of the original acid phosphatase activity of the yeast was recovered in the supernatant.

Attempts were made to prepare and purify cell walls to determine if the phosphatase were localized in the wall. Rupture of log-phase cells by 3 passages from $15 \cdot 10^3$ lb/in^2 in a French pressure cell (Aminco) followed by repeated centrifugation and washing, and finally centrifugation in a sorbitol gradient gave a good cell-wall preparation (judged microscopically). The preparation did not contain acid phosphatase. Breakage of cells with glass beads in an Omnimixer (Lourdes) or a Minimill (Gifford-Wood Co.) or sonication at 10 kcycles also liberated acid phosphatase in a soluble form. These experiments do not eliminate a localization of acid phosphatase on the wall, however, because its attachment to the wall could be quite labile.

Yeast protoplasts are capable of synthesizing acid phosphatase, and in the absence of a cell wall this newly formed phosphatase is found in the medium. Protoplasts were prepared from cells grown in phosphate. They were washed free of phosphate and incubated at 30° in phosphate-free medium containing 0.6 M KCl, $2 \cdot 10^{-3}$ M glucose, and $4 \cdot 10^{-2}$ M sucrose. The cell count was $4 \cdot 10^7$ per ml. Fig. 1 shows the production of acid phosphatase in the medium. In 3 h there was a secretion of about 4 units of acid phosphatase per 10^8 cells. There was no acid phosphatase on

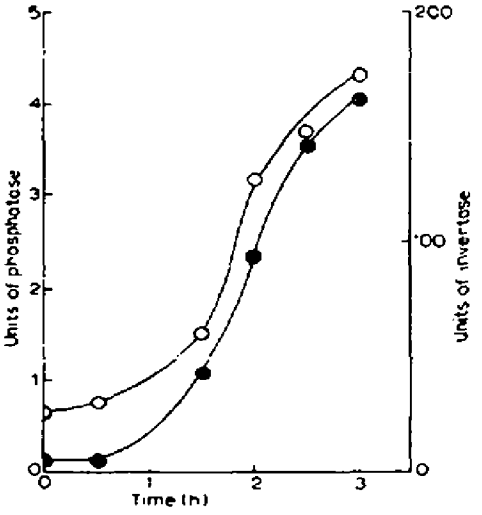

Fig. 1. Secretion of acid phosphatase and invertase by yeast protoplasts. ○ --○, phosphatase; ●—●, invertase. The units are μmoles/h/10^8 cells.

the surface of the protoplasts. The acid phosphatase inside the protoplast increased from 0.3 to 0.8 units/10^6 cells in 2 h and then the level dropped to 0.2 units at 3 h. The alkaline phosphatase inside the protoplast remained at 3.6 units/10^6 cells throughout the course of the incubation and none was found in the medium. The fact that no alkaline phosphatase was present in the medium confirms the view that the secreted acid phosphatase was not released by lysis of the protoplasts. For comparison the invertase synthesized and secreted into the medium is shown (Fig. 1). As invertase[6,7], the acid phosphatase of yeast is external to the cell membrane, and in the absence of cell wall newly synthesized enzyme is secreted into the medium.

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Separation of a Tribolium-protease inhibitor from soybeans on a calcium phosphate column

The preparation of a protein fraction (C_1) from soybean meal which inhibits growth and proteolytic activity *in vitro* of *Tribolium confusum* larvae and trypsin has been reported by LIPKE *et al.*[1]. It has also been found that C_1 inhibits α-chymotrypsin[2] and possesses a strong amylase activity[3]. The present study comprises an attempt to separate the Tribolium inhibitor of C_1 from the accompanying trypsin inhibitor and soybean amylase.

Proteolytic and inhibitory activity was determined by the casein digestion method[4]. Amylase activity was determined by the method of NOELTING AND BERNFELD[5] using the modified 3,5-dinitrosalicylic acid reagent. Larval enzyme solutions were prepared by dissecting out midguts of last-instar larvae. The midguts were then homogenized and centrifuged as described by BIRK AND APPLEBAUM[6]. Larval enzyme solutions were freshly prepared before each test. Trypsin and α-chymotrypsin were commercial crystalline preparations obtained from Worthington Biochemical Corporation. C_1 was prepared from ether-extracted soybean flour (Lincoln var.) according to LIPKE *et al.*[1].

An attempt to fractionate C_1 (14.4% N) on a DEAE-cellulose column resulted