Localization of Acid Phosphatase in Protoplasts from *Saccharomyces cerevisiae*

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The localization of acid phosphatase (EC 3.1.3.2) in secreting protoplasts prepared from *Saccharomyces cerevisiae* is reported for the first time. Using a Gomori technique we were able to show acid phosphatase at those organelles in the protoplasts which are generally involved in the processes of biosynthesis and secretion of glycoproteins in eukaryotic cells.

Under favorable conditions yeast protoplasts are able to secrete newly formed cell wall components, such as mannan, invertase (EC 3.2.1.26), and acid phosphatase (EC 3.1.3.2) (6, 10, 12, 23). In studies concerning the latter enzyme it has been shown that a small but reproducible amount of acid phosphatase is strongly bound to secreting protoplasts (23).

We investigated this enzyme fraction because it might have a function in the biosynthesis of the secreted acid phosphatase. About 70% of this protoplast-bound activity can be extracted with 1% Triton X-100 (TE fraction). In tracer experiments, using a radioactive amino acid, we provided evidence for the existence of a precursor-product relationship between the enzyme in this Triton extract and the secreted enzyme (3).

Whether this protoplast-bound acid phosphatase is localized within the plasmalemma or outside the protoplast was not yet clear. An answer to this question would provide us with more insight in the secretory process of the exoenzyme. We therefore decided to apply a cytochemical approach for the detection of acid phosphatase with the electron microscope. Günther et al. (8) and Bauer and Sigarlakie (2) demonstrated acid phosphatase in whole yeast cells. However, as far as we know this is the first time that this enzyme is demonstrated in yeast protoplasts.

**MATERIALS AND METHODS**

**Organism and cultivation.** Protoplasts, secreting acid phosphatase, were prepared as described previously (23).

**Electron microscopy.** Protoplasts were prefixed for 30 min in a mixture of 3% glutaraldehyde, 2.5% dimethylsulfoxide, and 12% (wt/vol) mannitol in 50 mM sodium acetate (pH 5.5). The fixed material was rinsed shortly in 12% (wt/vol) mannitol, 2.5% dimethylsulfoxide in 50 mM sodium acetate (pH 5.5).

After incubation for acid phosphatase activity the protoplasts were again fixed for 30 min in a mixture of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, 2.5% dimethylsulfoxide in 0.1 M sodium cacodylate (pH 7.4). Then the fixed material was rinsed thoroughly in the 0.1 M buffer, pH 7.4, and postfixed 30 min in 1% OsO4 in the same buffer. Fixation and washings were carried out at room temperature. The fixed material was dehydrated in graded acetone and embedded in Araldite. Thin sections were cut on a Reichert OMU 2 microtome. Sections were stained according to the method of Millonig (14). Both stained and unstained sections were studied. Electron micrographs were taken with a Philips EM 200 or a Philips EM 201 electron microscope.

**Cytochemical localization of acid phosphatase.** Acid phosphatase activities in the protoplasts were located by a modified Gomori (7) method using *para*-nitrophenylphosphate or *β*-glycerophosphate as a substrate. The prefixed protoplasts were incubated at room temperature for 30 min in 50 mM sodium acetate (pH 5.5), containing 2.3 mM leadnitrate, 8.2 mM substrate and 2.5% dimethylsulfoxide. The following control experiments were carried out: (i) incubation of nonsecreting protoplasts (protoplasts suspended in a medium containing 1 mM phosphate); (ii) incubation of protoplasts without substrate; and (iii) addition of an enzyme inhibitor, NaF, to the incubation medium.

**RESULTS**

The pH stability range of protoplast-bound enzyme is not very broad, namely, from pH 3.0 to 5.5 (3). Irreversible inactivation takes place at lower and higher pH. After 30 min of fixation with glutaraldehyde at pH 5.5 the remaining activity is about 75 to 80%. Fixation below pH 5.5 gave a poor preservation of the protoplast structure. Rinsing and incubation at pH 4.5 after glutaraldehyde fixation at pH 5.5 was unreliable for ultrastructure preservation. The preservation of some subcellular organelles, like
mitochondria, was rather poor due to the pH and the character of the buffer.

Lead phosphate precipitates were found in particular regions in protoplasts, which were incubated in a modified Gomori medium. It is observed in vesicles or small vacuoles (Fig. 1 and 2A), in the central vacuole (Fig. 1, 2A, and 3A), in flat vesicles beneath the plasma membrane (Fig. 1, 2A, 2B, and 3C), in the endoplasmic reticulum (Fig. 1 and 3A), sometimes in the nuclear membrane (Fig. 1), in Golgi-like structures (Fig. 2A), and on the surface of the protoplast. The same protoplasmic structures, but lacking a lead phosphate precipitate, are found in protoplasts, in which synthesis of acid phosphatase is repressed (Fig. 2C, 3B, 3D) or in protoplasts incubated without substrate or with enzyme inhibitor.

We found differences between the individual protoplasts with regard to extent and localization of acid phosphatase activity as visible by the presence of lead precipitate due to enzyme action. For instance, the central vacuole and the outer surface of the protoplast did not always show activity, nor did the nuclear membrane. To obtain an impression of the distribution of the acid phosphatase activity in the secreting protoplasts, a number of protoplasts were exam-

![Fig. 1. Unstained section of a secreting protoplast of Saccharomyces cerevisiae, incubated for acid phosphatase. ER, endoplasmic reticulum; FV, flat vesicles; NM, nuclear membrane; PM, plasma membrane; V, vesicles or small vacuoles; I, invagination. ×26,000.](image-url)
ined, and the number of times that a certain location was observed was noted (Fig. 4). About 10% of the protoplasts show no enzyme activity.

**DISCUSSION**

Considering the distribution of acid phosphatase activity in secreting protoplasts (Fig. 4), the enzyme appears to be located mainly within the protoplast. Some activity is found on the surface of protoplasts which is due either to absorption of enzyme from lysed protoplasts or to cell wall synthesis.

According to Bauer and Sigarlakie (2) acid phosphatase is located in the cell wall, in small vesicles or vacuoles, and in the nucleus of whole yeast cells. These results are in agreement with the observations of Günther et al. (8). We found acid phosphatase associated with the endoplasmic reticulum, the flat vesicles and sometimes with the nuclear membrane. Although Matile et al. (13) state that these flat vesicles belong to the endoplasmic reticulum we made a distinction between these structures, because we observed a difference in activity: only 14% of the

**FIG. 2. Unstained sections of protoplasts of S. cerevisiae.**

A, Derepressed protoplast lacking activity in the nuclear membrane (NM), endoplasmic reticulum (ER), and in the flat vesicles (FV). ×26,000. B, Detail of a derepressed protoplast incubated for acid phosphatase, showing fusion of a flat vesicle with the plasmalemma (arrow). ×58,200. C, Detail of a repressed protoplast, showing Golgi-like structure (G) lacking precipitate. ×33,600.
protoplasts show activity in both endoplasmic reticulum and flat vesicles. Hereward (9) described these flat vesicles as subsurface cisternae and related them with sites of protein synthesis. The endoplasmic reticulum plays a role in yeast cell wall synthesis (13), whereas in plant cells these subsurface cisternae are also involved in the synthesis of material outside the plasmalemma (18).

The endoplasmic reticulum is derived from the nuclear membrane (Fig. 2A arrow; see Matile et al. [13] for references). The enzyme

FIG. 3. Details of stained sections of protoplasts of S. cerevisiae. A, Derepressed protoplast showing activity in the endoplasmic reticulum (ER) and in the central vacuole (CV). ×52,000. B, Repressed protoplast. ×52,000. C, Derepressed protoplast, showing activity in the flat vesicles (arrows). ×52,000. D, Repressed protoplast; a flat vesicle (arrow) is shown. ×52,000.
activity associated with this membrane is probably not due to glutaraldehyde activation as has been suggested by Bauer and Sigarlakie (2), because in our experiments aldehyde fixation never exceeded 30 min.

We observed acid phosphatase in the central vacuole in about 50% of our active protoplasts, whereas Bauer and Sigarlakie (2) found no activity in this organelle. We were not able to exclude the possibility of intracellular degradation of acid phosphatase concomitant with its synthesis and excretion. The activity in the central vacuole, which is considered as a secondary lysosome (13), is in this respect very suggestive.

Since it is known that the central vacuole is divided into a number of smaller vacuoles during mitosis (13), a number of active small vesicles could be derived from the central vacuole in such a way. We, however, found them also occurring together with the central vacuole. At this moment we are not able to determine the function of the small vesicles.

We have found another characteristic structure in which acid phosphatase is located. This structure has some morphological resemblance with the Golgi apparatus (19, 20), whereas preliminary results indicate that thiamine pyrophosphatase (EC 3.6.1.1) activity, a marker for Golgi apparatus (5), is associated with the same structure. Since the occurrence of Golgi in yeast is controversial (1, 8, 13, 15, 16, 17) we tentatively describe this structure as Golgi-like. Further research is being carried out to elucidate this point.

The localization of acid phosphatase in the endoplasmic reticulum in the small vesicles or vacuoles, in a Golgi-like structure, and in flat vesicles underneath the plasmamembrane, agrees with the view that the exocellular yeast glycoproteins are synthesized following a pathway similar to that of glycoproteins of higher eukaryotes (4, 11, 21, 22, 23). Although we are not able to clarify the function and ontogeny of the diverse organelles with respect to glycoprotein synthesis, we can now conclude that the precursors of the exocellular acid phosphatase (3) are located within the plasmalemma.

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LITERATURE CITED