Digestion of Living Yeast Cells with Physarum polycephalum

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Abstract

On a mixed culture of Physarum polycephalum and Candida utilis, yeast cells ingested in the living plasmodium are digested, and remnants of the cell wall are found in the plasmodium in addition to cells in an almost intact form with intracellular highly dense substances. When growing yeast cells are treated with an extract from plasmodia the cell wall and intracellular substances are also disintegrated. The digestion, however, did not advance to produce spheroplasts or protoplasts under the condition employed in this experiment.

It is well-known that enzymes derived from some organisms are available for the lysis of yeast cell walls, and this subject have been discussed in a review by Phaff. Particularly, an enzyme preparation from snail was successfully applied to obtain the protoplast of yeast cell and made easy to observe fine structures of the yeast cell by electron microscope. Recently Rosness reported that the cellular slime mold, Dictiostelium discoideum, produced cellulolytic enzymes during the culmination and sorocarp stages of development. It has also been known for a long time that a slime mold ingests yeast cells. The present author made a mixed culture of Physarum polycephalum and Candida utilis, and examined whether or not the disintegration of yeast cells occured upon their ingestion in the growing plasmodium. Incubation experiment of the yeast cell with a crude enzyme solution extracted from the plasmodium was also carried out. The purpose of this paper is to know electron microscopically, the treatment with the digestive juice of the slime mold, Physarum polycephalum, is enable or not to lyse the yeast cell wall in the same manner as the sanil enzyme, and make easy to observe the intracellular fine structures.

Materials and Methods

Mixed culture of Physarum polycephalum and Candida utilis: The axenic plasmodium of P. polycephalum, developed from a strain of North Carolina Biological Association on a medium containing chicken embryo extract, was inoculated on the colony of C. utilis (HUT: 7526) previously cultured on an agar slant containing 3% malt extract (Difco) and 1% yeast extract (Daigo). The plasmodium grown in the dark at room temperature for 24 hours was fixed and embedded in order to observe the digestion of yeast cells in the plasmodium by electron microscope. For the survival test of yeast cells ingested in the plasmodium, a fresh liquid medium containing malt extract and yeast extract was added to a hyaline slime material isolated from the plasmodium.

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Preparation of digestive juice from the plasmodium: Plasmodia developed from the mixed cultures for about two weeks were homogenized in a cooled McIlvain buffer solution at pH 6.8 containing 0.5 M rhamnose and 0.01 M MgCl₂. The homogenate of 0.1 g of the wet plasmodium in 1 ml of the buffered solution was centrifuged at about 10,000 g for 10 min. The supernatant stored at −27° was used as a digestion medium without further treatment.

Digestion of yeast cells with the plasmodial juice: A roop of C. utilis cells growing on the slant was suspended in 1 ml of the plasmodial juice and incubated at 30° for one to two hours. As a control, yeast cells were alone incubated in the same buffered solution containing no plasmodial juice.

Preparation of specimen for electron microscopy: Both the materials obtained by the procedures above mentioned, i.e. the plasmodium grown on the mixed culture for 24 hours and the growing yeast cells treated with the plasmodial juice, were fixed with 4% glutaraldehyde in 0.1 M Soerensen's phosphate buffer at pH 7.0, postfixed with 1% osmium tetroxide in the same buffer, dehydrated with a graded aceton series, and finally embedded in epoxy resin. In order to examine components included in the plasmodial juice, several drops of 3% osmium tetroxide solution were added to 1 ml of the plasmodial juice. The resulting osmiophilic sediment was routinely dehydrated and embedded. The blocks were sectioned by Porter-Blum MT-1 microtome. After double-staining with uranyl acetate and lead citrate, sections were observed in an Akashi TRS-50E electron microscope.

Results and Discussion

Intact yeast cells: It is very difficult to observe fine structures of intact yeast cells with a routine thin sectioning technique such as glutaraldehyde and osmium tetroxide fixation and epoxy resin embedding, because the cytoplasm is insufficiently embedded and the fine structures are masked with a strongly stained material. The vague profil of intact C. utilis cells is seen in control cells treated with the buffer solution without the plasmodial enzyme (Fig. 5). While cytoplasmic organelles, even the nucleus or the mitochondrion, are not accurately indicated, almost amorphous and unstained cell walls are only distinguishable (Fig. 5).

Digestion of yeast cells in the growing plasmodium: Yeast cells in invaginations of the plasmodium show various figures from the almost intact cell to the remnant of cell wall (Figs. 1–3). The fact may be due to the difference in degree of influence with digestive enzymes on cell to cell in the growing plasmodium. In yeast cells thought to be weekly influenced (in a early stage of disintegration), intracellular substances of the yeast cell are still strongly stained (Fig. 1), and no intracellular structure is identified as in the case of intact cells. The cell wall, especially the outer layer of the wall is, however, distinguishable in highly dense state. The cell wall thought to be considerably influenced is distinguishable into two layers, but their boundary is not clear (Figs. 1–3). While the outer layer shows a compact fibrillar texture with a high electron density, the inner layer a less dense, coarse structure. The fibrillar texture of the cell wall may be unveiled, owing to dissolution of a ground substance. Penetration of fixatives and resin monomers also seems to be improved. Theses fibrils may correspond to fibrillar remnants of snail.
enzyme-treated cells of *Saccharomyces cerevisiae* etc. revealed through a freeze-etching method by Streiblova. The fibrillar texture is much more compact than a cell wall of *Saccharomyces carlsbergensis* treated with bacterial phosphomannase by McLellan, McDaniel and Lampen. In the living plasmodium, complete removal of fibrillar material as shown in a spheroplast formed by phosphomannase and snail enzyme is not recognized. In some yeast cells as shown in Figs. 1-3, intracellular membranous structures such as the plasma membrane, the endoplasmic reticulum, mitochondria, etc. are clearly seen. These figures are comparable with those of *Sac. cerevisiae* treated with snail enzyme described by Darling, Theilade, and Birch-Andersen. In these cells digestion by the growing plasmodial enzymes seems to proceed to make strongly stainable substances solute out of the cytoplasm. The plasma membrane detaches from the inside of the cell wall (Fig. 1, arrow) and extrusions of cytoplasm (Fig. 1, e) are seen in the gap between the plasma membrane and the cell wall. Scrolled remnants of disintegrated cell walls are seen in the plasmodium (Fig. 3). In these yeast cells thought to be strongly digested in the plasmodium, not only the cell wall but also the intracellular structures are lysed and the rest of cytoplasmic material is seen wrapped up by the scrolled cell wall remnant (Fig. 3). According to the results above mentioned, it is considered that, when *C. utilis* cells are cultured with the plasmodium of *P. polycephalum*, yeast cells are ingested and digested in the plasmodium. A hyaline slime material containing digested yeast cells is isolated from the plasmodium. Upon addition of a fresh medium to the slime material, however, some yeast cells grow again. These cells isolated in the slime should have been more or less affected by the plasmodial enzyme. As confirmed electron microscopically, the cell wall of the ingested yeast is disintegrated in the plasmodium. It seems likely that enzymatic damage such as the disintegration of the cell wall does not always inhibit the growth of the yeast cell.

*Digestion of yeast cells with the plasmodial juice:* The extracted plasmodial juice contains membranous structures and lipid granules (Fig. 4). In growing yeast cells treated with the juice at 30°C for two hours, the texture of the cell wall is fibrillar and the stainability of the wall seems to be increased (Figs. 5-7). The outer wall layer, about 300 Å thick, is strongly stained, and a fibrillar velvety texture is seen on its outer surface (Fig. 7). The inner wall layer, about 700 Å thick, appears to be made up of loose fibrils of random arrangement (Fig. 7). At the plug of the bud scar (Fig. 7), the outer layer is extremely thin, while the inner layer covering all over the plug is thick and denser. The fibrils in the inner layer of the plug arranged in parallel with the surface of the plasma membrane. The plasma membrane with many invaginations is as strongly stained as the fibrils in the digested cell wall. Furthermore, this enzymatic treatment seems to make the observation of intracellular membranous structures easier (Figs. 5-7), probably due to a change of cytoplasmic substance of high electron density. In these cells cytoplasmic organelles are clearly seen as in the cells thought to be moderately influenced in the growing plasmodium (cf. Figs. 1-3). This coincidence of the electron microscopic figures of yeast cells in the plasmodium with those treated with the plasmodial juice, may make more certain the consideration that the yeast cells are digested by enzymes in the plasmodium. In the nucleus (n) surrounded by the envelope, the nucleolus (nc) consisted of granules about 200 Å in diameter and fibrils occurs. A slightly dense zone in the karyoplasm suggests the chromatin. In the mitochondrion a few...
cristae are recognizable but their arrangement is rather obscure. In the cytoplasm closely packed ribosomes are seen (Fig. 7). Cytoplasmic structures might be suffered some change in the process of digestion, but the appearance of the nucleus and the mitochondrion and the size of the ribosome seems to be normal.

Protoplasts were obtained by the treatment of the yeast cell with snail enzyme. In the present investigation, an attempt failed to obtain the spheroplast or protoplast of C. utilis with the extracted plasmodial juice. The cell wall polymer, however, is obviously affected by the plasmodial juice, because the stainability of the cell wall is increased. These suggest that the plasmodial juice presumably contain similar enzymes as snail enzyme and the spheroplast or protoplast formation with the plasmodial juice of P. polycephalum may be possible under more advanced conditions.

References


Explanation of figures

Fig. 1. Part of a plasmodium of Physarum polycephalum ingested Candida utilis cells. Three yeast cells (cu) differ in digestion degree. Cell walls (w) are strongly stained, as a result of the digestion (cf. Fig. 5). In the central yeast cell (cu), cytoplasmic extrusions (e) are seen.

Figs. 2 and 3. Digested Candida utilis cells in plasmodial invaginations of Physarum polycephalum. A lysed plug of the bud scar (bs) (Fig. 2) and scrolled remnants of the cell wall wrapping up the disintegrated cytoplasmic substance are shown (Fig. 3).

Fig. 4. Osmiophilic components contained in the plasmodial juice of Physarum polycephalum. Membranous fragments (Mf) and lipid granules (L) are seen.

Fig. 5. Control yeast cells incubated in the buffered solution without the plasmodial juice. The cytoplasm is highly dense, whereas the cell wall is less dense.

Fig. 6. Candida utilis cells incubated with the plasmodial juice of Physarum polycephalum thought to be moderately digested.

Fig. 7. Details of a Candida utilis cell treated with the plasmodial juice. The cell wall (w) consists of fibrils. The arrangement of the fibrils at the plugs of bud scars (bs) are different from that at the other part (w). The plasma membrane is stained stronger than the other intracellular membranes. The nuclear envelope and the nucleolus (nc) are well seen, but the chromatin is obscure. The vacuole (v) includes membranous material.

Abbreviations used:
Physarum polycephalum (Iv = invagination of plasmodium, Mf = membranous fragment, L = lipid granule)
Candida utilis (cu = cell of C. utilis, bs = bud scar, e = cytoplasmic extrusion, m = mitochondrion, n = nucleus, nc = nucleolus, v = vacuole, w = cell wall)