ULTRASTRUCTURAL LOCALIZATION OF PHOSPHOHYDROLASES IN GAMETES, ZYGOTES AND ZOOSPORES OF ULVA MUTABILIS FØYN

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SUMMARY

Gametes, zoospores, and zygotes of the multicellular, green alga Ulva mutabilis showed acid phosphatase reaction product in Golgi vesicles and on the membrane lining the vacuole. In addition gametes and zoospores showed enzyme reaction product on the entire surface membrane including the flagellar membrane. The surface membrane enzyme activity disappears from the zygote shortly after copulation and at the same time lysosome-like bodies start to appear in the cytoplasm. No alkaline phosphatase activity could be detected. The distribution of acid phosphatase is discussed in relation to the events taking place during and shortly after fertilization.

INTRODUCTION

It has been long established (see for instance Förster & Wiese, 1955; Wiese, 1965) that gametes of green algae when shed from the gametangium and entering the freeswimming period of their life cycle, secrete a substance (gamon) which acts as a chemotactic substance enabling the gametes of different mating types to make contact. When this contact has been established, the plasma membranes disintegrate at the point of contact and the 2 cells rapidly fuse (Braten, 1971). The plasmalemma of the gamete thus plays an important role in the fertilization process and must contain highly specific recognition sites since gametes of identical mating type will not fuse. Identical gametes can be made to agglutinate by adding gamon extract to the medium, but fusion of the cells will not occur (Förster & Wiese, 1954).

The events following recognition and zygote formation such as fusion of the plasmalemma of the 2 gametes, absorption of the flagella through the cell surface (Braten, 1971), fusion of nuclei and the possible disintegration of one of the two chloroplasts (Braten, 1973), presumably require the presence of hydrolytic enzymes. An ultrastructural investigation of the distribution of unspecific acid and alkaline phosphatases in gametes and young zygotes was therefore undertaken.
MATERIALS AND METHODS

Algae were grown, and gametes, zygotes and zoospores were obtained as described in previous papers (Bråten, 1971; Bråten & Nordby, 1973). The following fixative, 4% glutaraldehyde in 0.2 M cacodylate buffer and 0.2 M sucrose, was diluted with an equal volume of seawater. The fixative had a pH of 7.4 and the fixation was carried out at 0 °C for 2 h. The specimens were then rinsed twice, each rinse lasting 1 h, in 0.2 M sucrose, 0.01 M cacodylate buffer and 10% DMSO (dimethyl sulfoxide) at pH 7.4 and 0 °C. The incubation was then carried out at 37 °C for 30–60 min. The incubation medium for unspecific acid phosphatase was that of the Gomori-type, using Na-β-glycerophosphate as substrate and lead as capture reagent. The medium was made 0.22 M with respect to sucrose and contained 10% DMSO (Brunk & Ericsson, 1972). Alkaline phosphatase detection was performed according to Mizutani & Barnett (1965) also using Na-β-glycerophosphate as substrate and cadmium as capture reagent. Control specimens were incubated either in medium lacking Na-β-glycerophosphate, or in complete medium containing 0.01 M NaF as enzyme inhibitor. After incubation the specimens were rinsed in equal volumes of 0.2 M cacodylate buffer and seawater, postfixed in 2% OsO₄ made up in equal volumes of 0.2 M cacodylate buffer and seawater. Dehydration took place in ethanol and Epon was used for embedding. The sections were observed in the electron microscope without further staining.

RESULTS

Acid phosphatase reaction product was found in the gametes, in the Golgi vesicles, in the vacuole, and on the entire surface membrane including the flagella (Figs. 1, 2). No difference in this distribution could be demonstrated between the 2 mating types.

After fertilization the picture changes as the staining for acid phosphatase disappears from the surface membrane of the zygote. Five minutes after fertilization some activity is still present, but 30 min after fertilization the activity is totally absent from the surface (Fig. 3). Golgi vesicles and the membrane lining the vacuole continue to show acid phosphatase reaction product, also in the zygote.

Electron-dense, membrane-bound bodies having a content which gives a positive reaction for acid phosphatase start to appear in the zygote approximately 30 min after fertilization (Fig. 3). No such lysosome-like bodies were found in the gametes.

The positive reaction for acid phosphatase on the surface of the gametes remains as long as the gametes are free-swimming. Gametes which were kept swimming for more than 24 h still showed staining of the surface of the membrane (Fig. 3 inset).

Ulva mutabilis has also a haploid, free-swimming stage, the zoospores, which develop into vegetative plants (gametophytes) without any fertilization process. These zoospores are morphologically similar to gametes, but have 4 flagella instead of 2 like the gametes. The same distribution of acid phosphatase staining as found in the gametes was found in zoospores.

Staining for alkaline phosphatase was tried to determine whether, for instance, the difference in enzyme distribution found between the gametes and zygotes was due to a pH shift. No staining for alkaline phosphatase could, however, be detected with the method used.
DISCUSSION

The presence of cell surface phosphatase activity has been previously demonstrated from reproductive cells of a marine organism. Anderson (1968) described the distribution of unspecific acid and alkaline phosphatase and ATPase from the periacroosomal plasmalemma of sea-urchin spermatozoa. Cell surface phosphatases are also known to occur on mammalian and human spermatozoa and Gordon (1973) has investigated the ultrastructural distribution of several phosphatases, including ATPase, of rabbit and human sperm heads. Five different phosphatases with different pH and substrate specificities have been isolated and chemically characterized from mammalian sperm (Gonzales & Meizel, 1973a, b).

In algae unspecific acid phosphatase has been demonstrated on the surface membrane of *Euglena* at specific sites on the pellicle (Sommer & Blum, 1965) in specimens where the enzyme activity was induced by growth in a phosphate-deficient medium. No constitutive acid phosphatase could be found at the surface of the organism and this enzyme is therefore thought to function as a non-specific hydrolase of organic phosphate in the absence of sufficient orthophosphate.

Hydrolitic enzymes on the surface of mammalian and invertebrate spermatozoa are believed to take part in the changes which occur in the spermatozoa prior to fertilization, i.e. fusion of the periacroosomal membrane with the acrosomal membrane and subsequent vesiculation and disappearance of these membranes (Anderson, 1968; Gordon, 1973). It is tempting to suggest that the presence of acid hydrolases at the surface of the gametes plays a role in the breakdown of the plasmalemma which takes place during the fertilization process. The absorption of the flagella into the cell immediately after zygote formation, a process which has been described elsewhere (Bråten, 1971) and which leads to fusion of the plasmalemma and the flagellar membrane, may also require the presence of surface hydrolytic enzymes. The disappearance of enzyme activity from the surface membrane shortly after copulation also indicates a connexion between the plasmalemma enzyme activity and fertilization.

Nothing is known about the uptake of nutrients by the gametes of *Ulva*. Gametes can be kept alive and swimming for several days if kept in the dark, which must mean that some uptake of nutrients takes place. It does not, however, seem likely that the sole function of the surface acid phosphatase should be in extracellular hydrolysis of phosphate from the medium, since the enzyme activity disappears shortly after the zygote is formed.

The possible role of enzymes as surface recognition sites should also be mentioned in connexion with this. Roth (1973) points out that not only antigen–antibody systems or lectins and oligosaccharides, but also enzyme–substrate reactions on cell surfaces may be involved in intercellular recognition. In *Ulva mutabilis* and in other isogamous algae highly specific surface recognition sites must exist. Only gametes of opposite mating type will fuse and copulation between gametes of different species has only been reported in rare cases (Wiese & Jones, 1963). Whether the acid phosphatase activity demonstrated here on the plasmalemma of the gametes has anything to do with intercellular recognition, is of course at present only speculation.
The presence of surface acid phosphatase on the plasmalemma of zoospores is not in agreement with the assumption that these enzymes function in the breakdown of the plasmalemma during the fertilization process since zoospores develop without any fertilization. Surface hydrolytic enzymes may, however, be required in the process of flagellar absorption also in zoospores.

The Golgi complex showed a high degree of acid phosphatase activity in all stages investigated. The role of the Golgi complex as a source of acid phosphatase in algae has been suggested previously (Brandes & Bertini, 1964). Investigations on other cell types point to the rough surfaced endoplasmic reticulum as a source of lysosomal enzymes and the Golgi complex as a site for concentration of the enzyme (Brandes, 1965). No evidence has been found during this investigation of the Golgi complex as a source for the surface acid phosphatase. No Golgi vesicles could be found in process of fusing with the plasmalemma, but this negative result may of course be due to lack of enough observations especially if the transport from Golgi to the surface is a rapid one. It is interesting to observe the almost immediate change in acid phosphatase distribution which takes place after copulation. Cell surface activity disappears and at the same time lysosome-like bodies appear in close connexion with the Golgi area (Fig. 3). It is likely that enzymes from these lysosomes function in the intercellular rearrangements which now take place in the zygote: disintegration of one of the chloroplasts, fusion of the nuclei, and breakdown of the absorbed flagella.

REFERENCES
Localization of phosphohydrolases in Ulva


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Fig. 1. Section through the anterior part of a gamete. Acid phosphatase reaction product can be seen in the Golgi complexes (g), on the plasmalemma (arrow), and on the flagellar membrane (double arrow). × 27 000.

Fig. 2. Section through the posterior half of a gamete. Note acid phosphatase reaction product in the vacuole (v), on the surface membrane, and on the flagella (f). c, chloroplast; n, nucleus. × 30 000. Inset: detail from surface of control specimen (gamete) incubated in a medium lacking Na-β-glycerophosphate. × 30 000.

Fig. 3. Section through a zygote fixed 30 min after copulation and incubated for acid phosphatase activity. In addition to Golgi cisternae (g), lysosome-like bodies (arrows) show enzyme reaction product. No activity can be detected on the plasmalemma (p). cw, cell wall; f, absorbed flagella. × 30 400. Inset: detail from gamete which had been kept swimming for 24 h before fixation and incubation for acid phosphatase activity. Golgi complex (g), vacuole (v) and plasmalemma show enzyme activity. × 25 500.
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