AN ULTRASTRUCTURAL STUDY OF THE RELATIONSHIP BETWEEN THE PLASMA MEMBRANE AND THE CELL WALL OF THE COENOCYTIC ALGA HYDRODICTYON AFRICANUM

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SUMMARY

A wall/plasma membrane unit was prepared from Hydrodictyon africanum by microdissection. A replica of the inner surface of the membrane was made and by freeze-fracture of the whole cell, 2 corresponding internal fracture faces were obtained. The large coenocytes of the alga were plasmolysed and the wall separated by cutting it away. Its inner surface was directly viewed in the electron microscope after shadowing with Pt/C. Particles were found on the outer half of the internal fracture face of the membrane which were oriented in the same 2 directions as the microfibrils laid down at the inner surface of the wall. No structures were found at the inner surface of the membrane. Some evidence was obtained for a structural connexion between the innermost layers of the wall and the plasma membrane.

INTRODUCTION

Bacteria and plant cells possess cell walls and the plasma membrane is thought to be involved in the production of at least part of this integument (Shore, Raymond & MacLachlan, 1975; Willison & Cocking, 1975; Northcote, 1969, 1974; Robinson & Preston, 1972; Preston, 1974; Brown & Montezinos, 1976). The alga Hydrodictyon africanum is especially suited to studies of the wall/plasma membrane relationship. The wall is extensive and its chemical composition has been determined (Northcote, Goulding & Horne, 1960), being made up of the sugars glucose and mannose probably β(1-4)-linked as glucomannans. The mature cells are large, up to 5 mm diameter, which enables micromanipulation to be used to obtain defined wall and membrane surfaces (Bailey & Northcote, 1976). In this study the results of both freeze-fracturing and direct replication are reported. Fig. 1 shows the possible fractures both within and around the plasma membrane which may occur during the process of freeze-fracturing (Moor & Mühlthaler, 1963; Branton, 1966; Hereward & Northcote, 1972, 1973); the nomenclature is according to Branton et al. (1975).

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MATERIALS AND METHODS

Direct shadowing of the inner surface of the cell wall

Mature coenocytes, cultured as described by Bailey & Northcote (1976), approximately 2–3 mm diameter, were plasmolysed in a series of concentrations of sorbitol buffered with 0.1 M sodium cacodylate, pH 7.2, by leaving the cells sequentially in 0.3, 0.4, 0.5 and 0.6 M sorbitol for 20 min. The retracted protoplast was fixed in 4 % glutaraldehyde, also buffered with 0.1 M sodium cacodylate, pH 7.2, the cell walls were cut open with a scalpel and the protoplast removed. The walls were washed in fresh buffer, dried on to a glass slide with their inner surface uppermost and shadowed in an AEI evaporator with platinum-carbon. The replica was cleaned in 72 % sulphuric acid for at least 12 h, followed by 2 washes in glass-distilled water, before viewing in an AEI EM6B electron microscope.

![Diagram](attachment://fig1.png)

Fig. 1. Diagram to show the possible fracture planes in and around the plasma membrane of Hydrodictyon africanum during freeze-fracture of the mature coenocytes. EF, internal face of the outer half-membrane of the plasma membrane revealed by splitting the membrane; ES, external surface of the plasma membrane; PF, internal face of the inner half-membrane of the plasma membrane revealed by splitting the membrane; PS, internal surface of the plasma membrane; Wi, inner surface of the cell wall; Wo, outer surface of the cell wall.

In Figs. 2–8 the circled arrow on each photograph indicates the direction of shadow.

Fig. 2. The inner surface of the cell wall of *H. africanum*. The wall was prepared by microdissection and shadowed with Pt/C. Microfibrils oriented in 2 major directions are clearly seen. Aggregates scattered over the wall surface are visible. x 38,000.

Fig. 3. The inner surface of the cell wall of *H. africanum*. The cells were fixed with glutaraldehyde, infiltrated with 30 % glycerol and freeze-fractured. The replica was made of W/Ta. Both microfibrils and aggregates are visible. x 55,000.

Fig. 4. A surface exposed by freeze-fracture of a layer within the wall. The replica was made of W/Ta. The microfibrils in these inner layers are still oriented in 2 major directions and are interwoven with one another. x 80,000.
Structure of the plasma membrane of H. africanum
**Freeze-fracture microscopy**

Mature coenocytes, approximately 2–3 mm diameter, were fixed in buffered 1 % glutaraldehyde solution (0·1 M sodium cacodylate, pH 7·2) for 1 h. Fixation was completed in 4 % glutaraldehyde in 0·1 M sodium cacodylate, pH 7·2 (3 changes, overnight). The coenocytes were infiltrated with 30 % glycerol (3 changes, overnight), and rapidly frozen in liquid freon for 20 s before rapid transfer to liquid nitrogen. The specimens were then mounted in a Balzers BA 360 freeze-etch machine and exposed to a vacuum lower than 5·3 × 10⁻⁸ N m⁻¹ (4 × 10⁻⁸ torr) at −100 °C. Fracturing was performed at −100 °C, followed by shadowing of the fractured surfaces using a tungsten-tantalum evaporator, with overlap-shadowing with carbon (Neushul & Northcote, 1976).

The tantalum-tungsten shadowing technique produces films without detectable crystallites. However, the acids used to clean the replicas dissolve the tungsten and oxidize the tantalum, so that this potentially high-resolution shadowing method is not held to be as acceptable and is not as widely used as the traditional platinum-carbon method. In attempting to minimize damage to the replica, the cleaning process was carried out in a well-slide with a coverglass bottom in such a way that the bulk of the material being cleaned from the replica could be observed during the cleaning process with an inverted microscope equipped with phase optics. The replica was cleaned for a few hours in 30 % chromic acid, followed by 2 washes in glass-distilled water, before viewing in the electron microscope.

**Preparation and shadowing of the wall/plasma membrane unit**

Micromanipulation of the coenocytes was performed to yield the wall with its adhering plasma membrane (Bailey & Northcote, 1976). The wall/plasma membrane units were rinsed with distilled water before being picked up on an electron-microscope grid with the inner surface of the preparation uppermost. Excess liquid was carefully removed before freezing the specimen in liquid freon. The specimen was mounted in the freeze-etch apparatus, exposed to high vacuum and allowed to deep etch continuously at a vacuum lower than 5·3 × 10⁻⁸ N m⁻¹ for 20 min. Replication was performed as described above without prior fracture and the replica viewed in the electron microscope.

**RESULTS**

Using micromanipulative techniques it was possible to remove the protoplast of the mature coenocytes of *H. africanum* and directly observe the inner wall surface (Fig. 1, surface Wi; Fig. 2) which lay adjacent to the plasma membrane. The wall possessed numerous microfibrils, about 7·5 nm in diameter, apparently deposited in 2 main directions almost at right angles (85–90°) to each other. In addition, numerous aggregates, about 4–7 times the width of the microfibrils, were scattered over its surface. The microfibrils did not appear to terminate at the aggregates. The aggregates must either have represented material which originally lay between the wall and the plasma membrane, or discrete areas of membrane which had been pulled out of the plasma membrane during plasmolysis and subsequent fixation.

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**Fig. 5.** A fracture of the plasma membrane to expose an internal face (PF). The replica was made of W/Ta. This face has a relatively low density of membrane-associated particles and bears numerous protrusions. × 60 000.

**Fig. 6.** A fracture of the plasma membrane to expose the corresponding internal face (EF) to that shown in Fig. 5. The replica was made of W/Ta. The underlying wall can be seen in the right-hand corner of the picture. The membrane face carries a high density of particles and these are aligned in the 2 directions of the microfibrillar orientation. The membrane face bears numerous depressions. × 58 000.
The freeze-fractured faces of the plasma membrane of *H. africanum* possessed a very uneven aspect with numerous protrusions and depressions (Figs. 5, 6). The smooth appearance of these protrusions (Fig. 5) suggested that they represented distortions imposed upon the membrane by structures either above or below it and not heterogeneities within the membrane itself. Two distinct surfaces were apparent, one possessing a high density of membrane-associated particles (5-7 nm) (Fig. 6), the other a lower density of membrane-associated particles (Fig. 5). The particles differed in appearance between the 2 faces. The surface with a high density of particles had depressions, while the surface with a lower density of particles contained protrusions. Where surfaces of the plasma membrane occurred adjacent to wall fractures (Fig. 6), it was possible to identify the surfaces with high particle densities as EF (Fig. 1) and the surfaces with low particle densities as PF. The particles of the EF were aligned, closely following the direction of the microfibrils in the adjacent wall areas (Fig. 6), while those of the PF were more randomly arranged (Fig. 5). The inner surface of the plasma membrane (PS), viewed without fracturing, possessed no obvious particulate substructure. Sheets of plasma membrane were obtained adhering to the inner surface of the wall (Figs. 7, 8). Certain areas of the plasma membrane had vesiculated, revealing the microfibrils of the inner (Wi) surface of the wall beneath. However, there did not appear to be any distinct structures on the inner surface of the plasma membrane which reflected the orientation of the microfibrils in the adjacent wall areas. In certain areas of the membrane's inner surface (PS), the membrane bore aggregates of similar size to those observed on the inner surface of the wall, while in other areas it was smooth (Fig. 8). During freeze-fracturing of the whole cells, the plane of fracture occasionally travelled along the inner surface of the wall, revealing a loose arrangement of microfibrils immediately adjacent to the plasma membrane (Fig. 3) as distinct from the more compact arrays within the wall (Fig. 4). The appearance of the inner surface of the wall observed during freeze-fracture closely resembled that of the dried inner wall surface prepared by microdissection (Fig. 2), although the microfibrils themselves appeared to be more projecting, perhaps being lifted out of the plane of the inner wall surface by the knife during fracturing. Fractures within the wall itself often revealed microfibrils, and the orientation of the interweaving microfibrils (Fig. 4).

Fig. 7. The inner surface of the plasma membrane (PS) revealed by microdissection and freeze-etching without fracturing or infiltrating with glycerol. The replica was made of W/Ta. The membrane is present as a sheet adhering to the inner surface of the wall, which can be seen beneath the membrane where the membrane has torn away or vesiculated. Some areas of the internal surface carry aggregates which are of a similar size to those seen on the inner surface of the cell wall (Fig. 2). × 28,000.

Fig. 8. The inner surface of the plasma membrane (PS) revealed by microdissection and freeze-etching without fracturing or infiltrating with glycerol. There are very few particles visible at this surface of the membrane. In the top right-hand corner the membrane has come away revealing the microfibrils of the wall underneath. × 52,000.
DISCUSSION

The differing morphology of the internal fracture faces (PF and EF) within the plasma membrane and the unfractured inner (PS) surface of the membrane revealed by microdissection shows the absence of clearly defined membrane-associated particles at the unfractured surface. Since glycerol impregnation was used for studies only of the whole cells, the smoothness of the inner surface (PS) of the plasma membrane cannot be due to glycerol concealing the presence of the particles. Also, the considerable differences both in particle size and distribution between the 2 internal fracture faces of the membrane suggests a clear differentiation between the 2 halves of the bimolecular leaflet, with ordered particulate organization on the EF and more random particulate distribution on the PF. The particles of the EF follow the orientation of the microfibrils of the adjacent wall layers, which suggests a structural connexion between the outer leaflet of the plasma membrane and the microfibrils of the innermost layer of the wall. However, it is not possible to correlate the biochemical role of these membrane-associated particles with microfibril assembly in _H. africanum_, as Brown & Montezinos (1976) have done with _Oocystis_, since the microfibrillar arrays are very much less complex in _Hydrodictyon_ than _Oocystis_ and would presumably involve a less highly differentiated assembly process.

The ordered arrangement of the particles on the internal fracture face of the membrane (EF) had no corresponding structures which related to their orientation and hence that of the microfibrils, on the inner surface of the plasma membrane (PS). There was therefore no evidence that the microfibrillar direction was controlled from the cytoplasm by structures applied to the membrane. However, the absence of organized material at the surface of the membrane may be because it was removed during the syringeing which was used to isolate the wall/plasma membrane unit.

The aggregates on the inner surface of the cell wall corresponded in size and general distribution with the depressions and protrusions seen at the fracture faces of the membrane and with aggregates found at the inner surface of the plasma membrane. Thus there was a possible connexion between the innermost layers of the wall and the plasma membrane even at the inner surface.

REFERENCES


Structure of the plasma membrane of H. africanaum


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