THE ISOLATION OF PLASMA MEMBRANES FROM THE CELLULAR SLIME MOLD *DICTYOSTELIUM DISCOIDEUM* USING CONCANAVALIN A AND TRITON X-100

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1. Introduction

Plasma membranes may be readily isolated from non-nucleated erythrocytes in hypotonic media [1]. However, nucleated cells possessing many intracellular structures must first be disrupted and the resulting vesicles of cytoplasmic and plasma membranes separated by exacting centrifugation schemes [2]. Methods of disruption resulting in identifiable larger fragments [3,4] include treatments thought to strengthen or stabilize the surface structure [5].

We have been seeking a rapid method to provide high yields of plasma membrane from *Dictyostelium discoideum* cells. We have unsuccessfully tried a number of techniques including zinc ions [5], borate [6] and an aqueous two-phase polymer system [7]. Fractions enriched in plasma membrane vesicles can be obtained using Potter-Elvehjem homogenizers [8] or by freezing and thawing in liquid nitrogen [9], but require time-consuming differential and density gradient centrifugation.

The present note describes a method we have developed for rapid isolation of plasma membrane in high yield using concanavalin A (ConA) and Triton X-100.

2. Materials and methods

D. discoideum cells (Ax3, from W. F. Loomis Jr.), grown in HL5 medium [10], were washed and resuspended in distilled H_2O (5×10^6 cells/ml) and shaken in an Erlenmeyer for 10 min. (Cell rupture was better and clean ghosts more consistently obtained following this pre-treatment). The cells are sedimented and resuspended in PDF solution (pH 6.5) [11] to a concentration of approx. 5×10^7 cells/ml. ConA (Calbiochem) was added to a final concentration of 100 μg/ml. After 2–5 min the suspension was diluted and washed (× 2) with 0.1 M Tris-HCl (pH 8.5) by centrifuging at 300 *g* for 1 min. The cells were resuspended in Tris-HCl buffer ($2–5 \times 10^7$ cells/ml) and Triton X-100 added to give a final concentration of 0.2% (v/v). The suspension was shaken on a Vortex mixer (5 sec) and a drop was examined under the microscope to ensure the majority of cells were ruptured. (Further Triton X-100 may be added if necessary.) The homogenate was diluted with Tris-HCl buffer and centrifuged at 300 *g* (10 sec) to remove whole cells. The supernatant was centrifuged at 1700 *g* (2 min), resuspended and washed at least twice. The pellet was examined for whole cells and, when necessary, could be treated again with Triton X-100. The final pellet was used for electron microscopy, gel electrophoresis and enzyme determinations.

Alkaline and acid phosphatase, β-*N*-acetylglucosaminidase were measured as previously described [8,12], and cAMP phosphodiesterase spectrophotometrically according to Yanagisawa et al. [13].

3. Results

3.1. *Phase-contrast microscopy*

The method can be conveniently monitored with a phase-contrast microscope (fig.1). The evaluation of ConA concentrations was carried out by placed ConA-treated cells on a slide and adding a drop of 10% (v/v) Triton X-100 beside the coverslip. If the ConA concentration was optimal the aggregates of cells were

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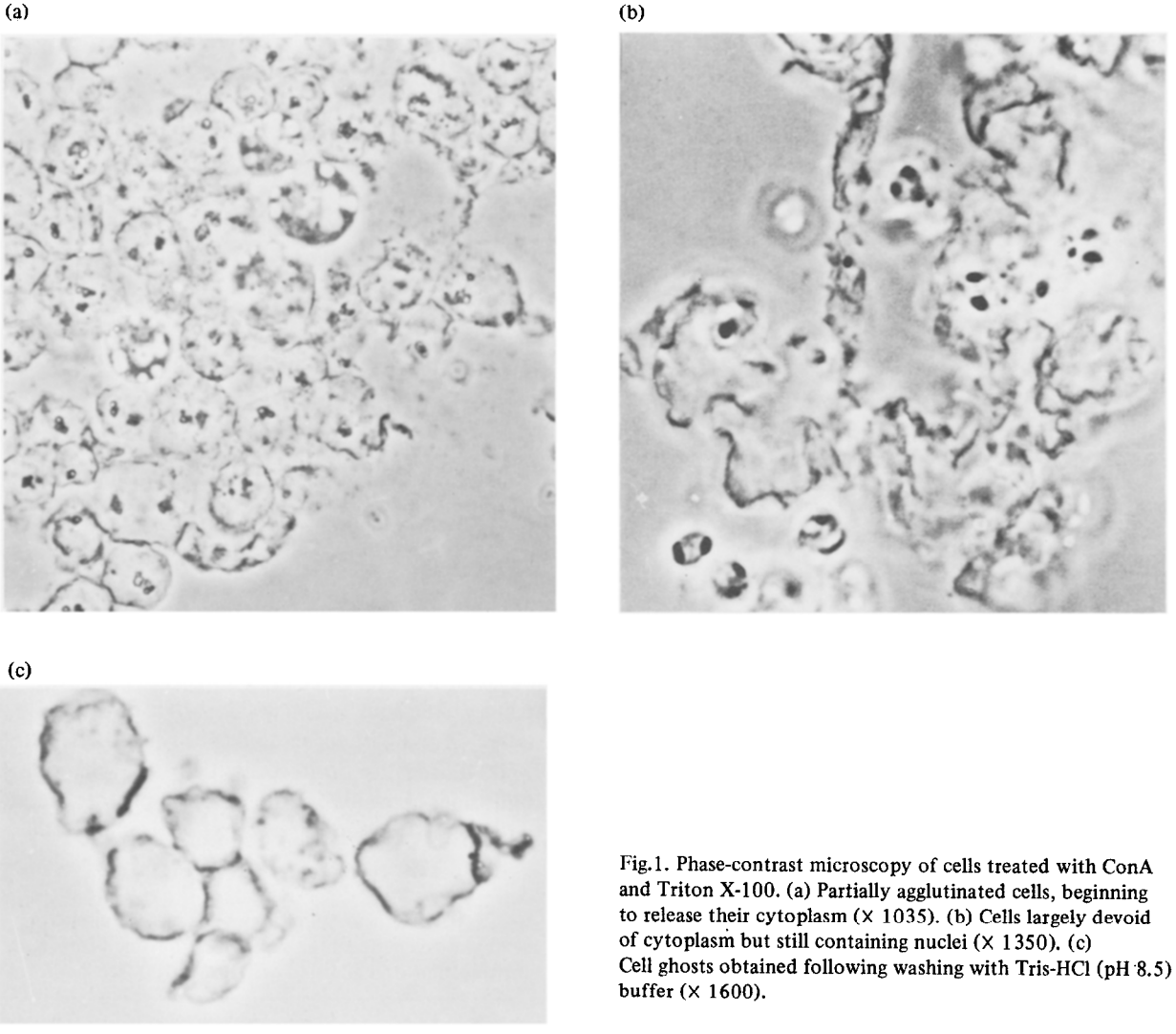

Fig.1. Phase-contrast microscopy of cells treated with ConA and Triton X-100. (a) Partially agglutinated cells, beginning to release their cytoplasm (× 1035). (b) Cells largely devoid of cytoplasm but still containing nuclei (× 1350). (c) Cell ghosts obtained following washing with Tris-HCl (pH 8.5) buffer (× 1600).

seen to release their contents and cell 'ghosts' remained (fig.1). If the concentration was too high the contents of the cells were incompletely released, whereas if too low, fewer ghosts were observed.

The ghosts were remarkably resistant to Triton X-100 and concentrations of up to 2% (v/v) were unable to solubilize them.

When the entire procedure was carried out in PDF solution (pH 6.5) the membranes were contaminated with nuclei which remained partly within the ghosts themselves (fig.1b). The use of Tris-HCl (pH 8.5) for washing overcame this problem (fig.1c).

3.2. *Electron microscopy*

The preparations contained large fragments of plasma membranes with the typical trilamellar structure [14] (fig.2). Two membranes were very often joined, presumably due to ConA binding (fig.2b). The preparation contained no recognisable contaminants.

3.3. *Effects of other lectins and detergents*

Wheat germ agglutinin and soya bean agglutinin were unable to reproduce the effects of ConA. Phytohemagglutinin-M (Calbiochem) and leuco-

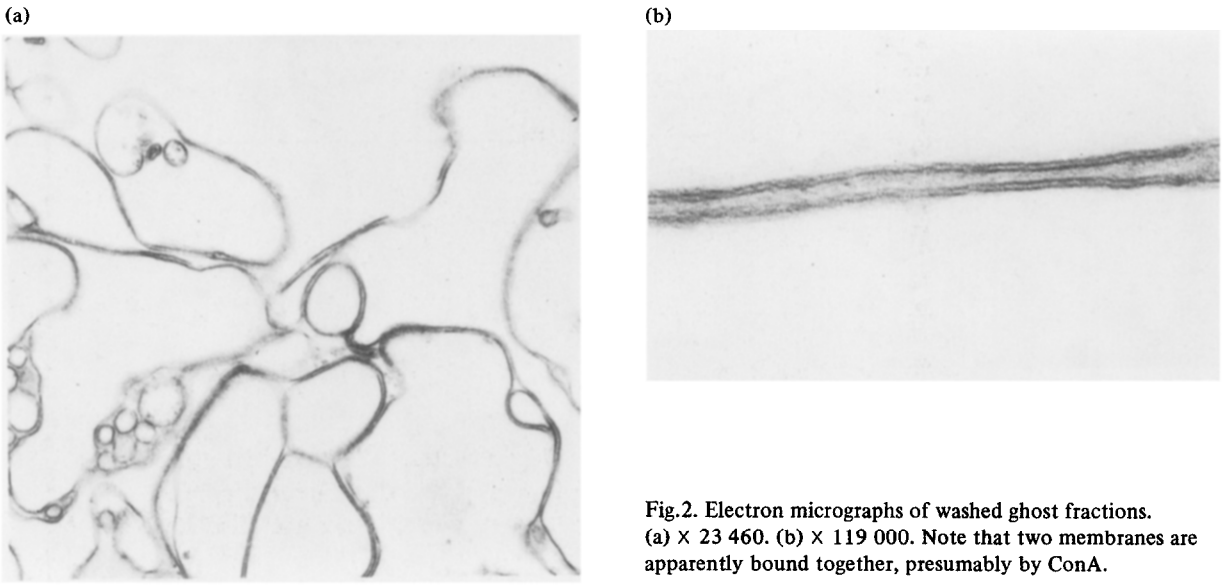

Fig.2. Electron micrographs of washed ghost fractions. (a) × 23 460. (b) × 119 000. Note that two membranes are apparently bound together, presumably by ConA.

agglutinin (Pharmacia) were more successful; however, the critical concentrations lay within a narrower range than ConA and careful pre-testing of each new batch of cells was necessary. Deoxycholate and SDS added to ConA treated cells produced 'ghosts' which were then rapidly dissolved. Cemulsol NPT, Kyro, EOB, Brij 38 and 56 only partly released cell contents.

3.4. *Enzyme activities*

Alkaline phosphatase is a marker enzyme for plasma membrane of *D. discoideum* [9] and is located, in part, on the outer surface [12]. Some of the cAMP phosphodiesterase activity is also surface associated [15]. The isolated plasma membrane demonstrated relatively high specific activities of both these enzymes (table 1). β-*N*-Acetylglucosaminidase and acid phosphatase, enzymes associated with phagocytic vacuoles ([12], in preparation), were not detected.

The plasma membrane fractions may occasionally be contaminated by whole cells, and this can be rapidly checked by measuring catalase activity. If activity is detected the preparation is impure and must again be treated with Triton X-100.

ConA treatment does partially prevent the release of alkaline phosphatase from the plasma membrane caused by Triton X-100 treatment (table 2). Some activity (3.5% of total) remains in the 700 *g* fraction from untreated cells, ConA treatment increasing the amount to 10.5%. This amount remains constant irrespective to Triton X-100 concentration. Total sedimentable activity (30 000 *g*, 2 h) is 50% for untreated and 67% for treated cells when 0.2% Triton X-100 is used, but drops to less than 30% with concentrations above 0.5%. The acid phosphatase present in the 700 *g* fraction was approx. 2.5% of the total

Table 1
Specific activities of enzymes in a washed plasma membrane fraction and the supernatant obtained from first centrifugation after addition of Triton X-100 (see Methods)

Enzyme	Washed plasma membrane	Supernatant
Alkaline phosphatase	1.31	0.06
cAMP phosphodiesterase	24.6	16.8
β-N-acetyl-glucosaminidase	n.d.	0.842
Acid phosphatase	n.d.	0.306

Alkaline phosphatase, β-*N*-acetylglucosaminidase and acid phosphatase expressed as μmol p-nitro-phenol × mg protein^{-1} × h^{-1} and phosphodiesterase as μmol cAMP × mg protein × min.
n.d. = not detectable.

Table 2
The effects on ConA treatment on the specific activity of enzymes present in a fraction sedimenting at 700 *g* (60 sec) after treatment with different Triton X-100 concentrations

Triton X-100 concentration	Control cells		ConA-treated cells	
	Alkaline phosphatase	Acid phosphatase	Alkaline phosphatase	Acid phosphatase
0.2%	0.176	1.23	0.564	1.10
0.5%	0.183	1.26	0.604	1.12
1.0%	0.174	1.16	0.535	1.15

Whole cells previously removed by centrifugation at 300 *g* (10 sec). Specific activities are μmol *p*-nitrophenol × mg protein^{-1} × h^{-1}.

activity and was not increased as a consequence of ConA labelling (table 2).

Although ConA apparently facilitates the retention of alkaline phosphatase on plasma membranes (approx. 7% of total activity) considerable activity is probably released. However, the amount is difficult to estimate as it is unknown what proportion of alkaline phosphatase is associated with plasma membrane.

4. Discussion and conclusions

The plasma membrane of cellular slime mold cells is normally very sensitive to Triton X-100, and we have utilized this to isolate intact organelles from cells ruptured with low concentrations of detergent [8,16]. However, the binding of ConA to the outer surface of the membrane, and possibly also lectin-lectin binding, stabilizes the membrane structure and protects it from Triton X-100. The detergent apparently punches holes in some parts of the membrane, allowing cell contents to be dissolved and released.

If cells are first treated with Triton X-100 after ConA caps have been formed, only the caps are isolated. The remainder of the membrane, which lacks ConA binding sites, is dispersed. Unpublished work with plant protoplasts also suggests the method is not applicable to cells having a low concentration of lectin binding sites.

Although the isolated plasma membrane appears morphologically intact, proteins are certainly lost during the preparation. The extent of this loss is not yet clear, but it may be that peripheral proteins are removed whereas integral (structural) proteins are largely left intact. SDS gel electrophoresis of the isolated plasma membrane has shown that eight major proteins (two attributable to ConA) and several minor proteins are present (paper in preparation). The most prominent protein has been identified as actin. It is notable that the three 'spectrin' proteins which may be 'erythrocyte actomyosin' are peripheral proteins associated with the cytoplasmic face of the plasma membrane [17].

We are at present comparing the preparations with plasma membranes isolated by other procedures and also applying the method to better characterized systems (e.g. erythrocytes).

The ConA–Triton technique should be applicable to many types of cells, permitting rapid plasma membrane isolation and also characterisation of membrane fragments enriched in lectin sites (e.g. caps).

During the course of this work, Scarborough [18] published a method whereby the plasma membranes of a wall-less mutant of *Neurospora crassa* are treated with ConA prior to homogenization. This protected them from fragmentation and vesiculation. *D. discoideum* cells treated with ConA, however, cannot be adequately lysed by mechanical homogenization.

Binding of Concanavalin A to the surface of cells renders the plasma membrane resistant to Triton X-100 but the cytoplasmic contents are solubilized by the detergent. The resultant cell ghosts can be purified by washing and low speed centrifugation. The isolated plasma membranes appear clean and intact in the electron microscope and carry high specific activities of alkaline phosphatase and cAMP phosphodiesterase. The lectin-detergent method may

provide a means for the rapid isolation of plasma membranes from many cell types.

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