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Electrolyte induced vesiculation of MA_{782} mice mammary adenocarcinoma ascites cells

Tan Zheng[1], Fang Shi-guo[2] and Liu Shu-sen[1]

[1]*Department of Cell Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, P.R. China* and [2]*Department of Virology, Wuhan University, Wuhan Hubei, P.R. China*

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MA_{782} ascites cells were found to vesiculate when incubated in electrolyte solutions and release giant plasma membrane vesicles with diameters ranging from 5 μm to 15 μm. The vesicles, generated from the plasma membrane and free of cellular organelles, as visualized by microscopy and marker-enzyme analysis, could be proposed as a useful model membrane system.

Membrane vesicle; Plasma membrane; Ascites cell

1. INTRODUCTION

Because of the complexity of the structure and components of nucleated cells, methods for obtaining plasma membranes have been complex, lengthy and the membranes obtained have often been small, submicroscopic vesicles or fragments [1,2]. Recently, we observed that MA_{782} ascites cells produced giant plasma membrane vesicles when incubated in electrolyte solutions. The vesicles, with diameters ranging from 5 μm to 15 μm, were free from cellular organelles and were easy to purify. In this report, we describe the generation and isolation of giant vesicles, and provide the results of preliminary analysis.

2. MATERIALS AND METHODS

The MA_{782} mice mammary adenocarcinoma ascites cell line was provided by the Department of Pharmaceutics, Wuhan Medical University and maintained by weekly intraperitoneal inoculation of parent cells into female mice of the Kueng Min White strain. The cells for experiments were harvested 6–7 days after inoculation.

2.1. *Vesiculation*

Unless otherwise indicated, fresh ascites cells were collected and washed at 4°C with Tris-HCl buffered saline (141 mM NaCl in 10 mM Tris-HCl buffer, pH 7.4) to remove erythrocytes and soluble contaminates. More than 95% of the cells were vital as examined by a Trypan blue exclusion test. The cells were then suspended in a 10 × volume of the same saline and maintained at 4°C for 4–8 h during which vesiculation took place. After incubation, the supernatants were removed and the sedimentary cells were resuspended in a 5 × volume of Tris-HCl buffered saline (105 mM NaCl in 50 mM Tris-HCl buffer, pH 7.4) and then centrifuged at 60 × *g* for 3 min. The 'mother cells' and the undetached vesicles were spun down to the bottom by centrifugation. The detached vesicles remained in the supernatant and were collected for subsequent analysis.

Correspondence address: T. Zheng, Department of Cell Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, P.R. China

2.2. *Electron microscopy*

For ultrathin sections, sample pellets were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer (PBS), pH 7.4 and subsequently in 1% OsO_4 in PBS, dehydrated in a series of ethanol, embedded in Spurr resin, and cut on a LKB 2088 Ultratome. Sections were stained with uranyl acetate and lead citrate. For freeze fracture, the sample pellets were fixed in 2.5% gluteraldehyde in PBS, submerged in 30% glycerol in PBS, frozen in liquid nitrogen, fractured and replicated on a Balzer's BAF-400D apparatus. Carbon-platinum replicas were digested in hypochlorate and washed in double-distilled water. For scanning electron microscopy, a drop of sample suspension was placed on a coverslip pretreated with 1.0% poly-L-lysine and fixed in 2.5% gluteraldehyde in PBS and subsequently in 1% OsO_4 in PBS, dehydrated in ethanol series and critical-point-dried in liquid CO_2. The coverslip was vacuum-coated with carbon and gold.

2.3. *Enzyme assay*

Spectrophotometric measurements for ouabain-sensitive Na^+,K^+-ATPase, NADH dehydrogenase, lactic dehydrogenase were performed according to the methods described by Schoner et al. [3], Hochstadt et al. [4], and Burgmeyer and Bernt [5], respectively. The specimens of vesicles and cells were counted and measured in diameter under an optical microscope before enzyme activity measurement. The specific enzyme activity of vesicles was defined as:

$$\frac{(A_v/S_v)}{(A_c/S_c)} \times 100$$

where A_v and A_c are the rates of decrease in absorbance with respect to vesicles and cells, S_v and S_c the total surface area of vesicles and cells, respectively, in the case of Na^+,K^+-ATPase, or the number of vesicles and cells in the case of NADH dehydrogenase, or the total internal volume of vesicles and cells in the case of lactic dehydrogenase.

3. RESULTS

3.1. *Vesiculation*

As monitored under an optical microscope, almost

all cells underwent vesiculation when incubated in Tris-HCl buffered salines of NaCl, KCl, $(NH_4)_2SO_4$ at a series of temperatures of 4, 10, 25 and 37°C. When isotonic saline was used, the intracellular organelles gradually condensed around the nuclei located at one pole of the cell, resulting in the formation of 'dumbbell' shaped cells with an asymmetric distribution of intracellular contents. The vesicles were later released from the 'mother cells' when the connection grew weaker. When hypertonic saline, for instance, 300–3000 mM NaCl or 300–1000 mM KCl was supplied, the cells became crenated immediately because of loss of intracellular water and then gradually swelled up producing vesicles more rapidly than in isotonic saline. The higher the concentration of saline used, the more rapidly the vesiculating process. No vesiculation was observed when cells were incubated in non-electrolyte solutions, for instance, of glucose, glycerol and sucrose.

3.2. *Morphological characterization*

The vesicles, under an ordinary optical microscope, were shown to be transparent circular rings in sharp contrast to the 'mother cells' which were filled with condensed organelles (Fig. 1A,B). Under the phase-contrast microscope, the vesicles did not show any internal structure (Fig. 1C,D,E). A fine corrugated surface similar to that of erythrocyte ghosts was observed. Fig. 3 gives the diameter distribution of cells before incubation and of vesicles after a one-step centrifugation. A considerable amount of vesicles around 15 μm in diameter preferably went down to the bottom during centrifugation. Electron micrographs of thin sections show that the vesicles are free from organelles and the main content is likely to be glycogen granules. Intracellular organelles were condensed around the nuclei in 'mother cells' from which vesicles were released (Fig. 2A,B). The membrane structure of vesicles was con-

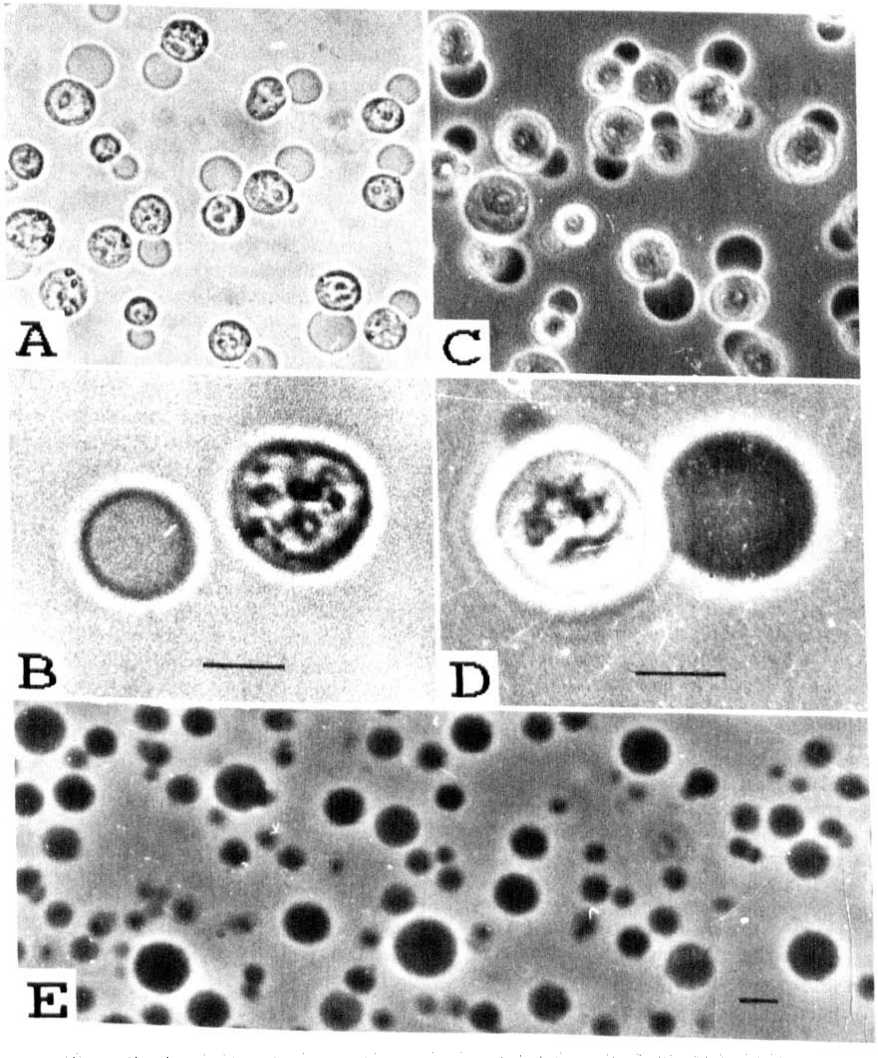

Fig. 1. Vesiculation of MA_{782} ascites cells observed under an optical microscope. Cells were incubated in 141 mM NaCl in 10 mM Tris-HCl buffer (pH 7.4). (bar = 10 μm). A. Vesicle-bearing cells. B. A released vesicle and its 'mother cell'. C. Vesicle-bearing cells (phase-contrast). D. A vesicle-bearing cell (phase-contrast). E. Vesicles purified by 3 min centrifugation at $60 \times g$ (phase-contrast).

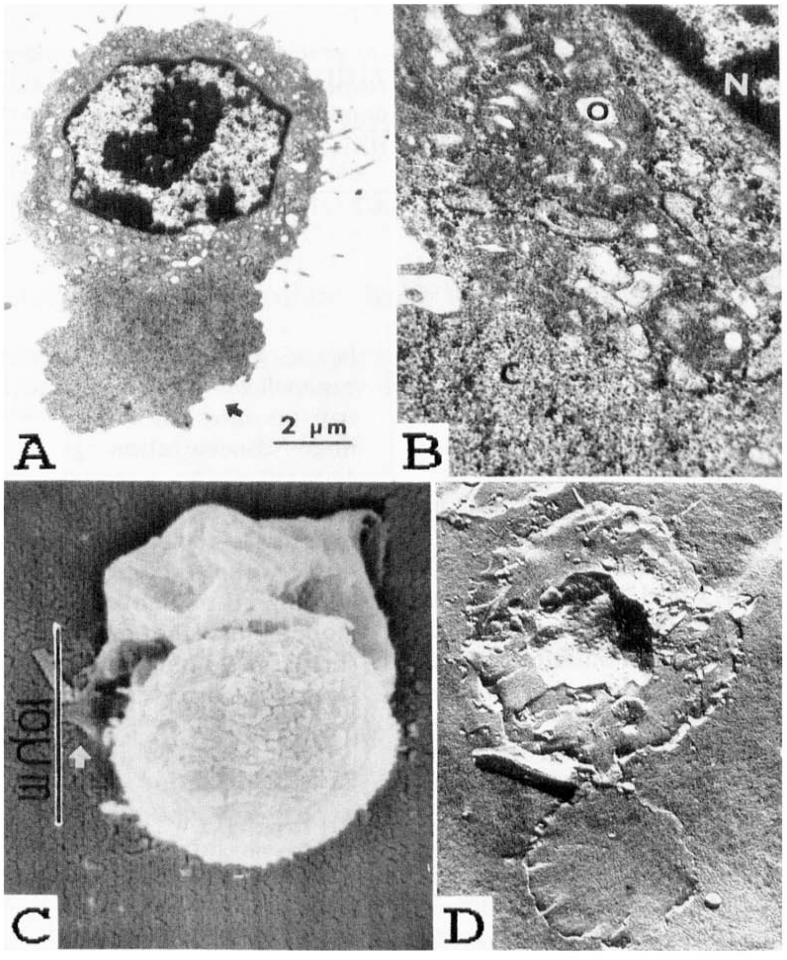

Fig. 2. Electron micrographs of giant plasma membrane vesicles from MA_{782} ascites cells. (A,B) Ultrathin section of a vesicle-bearing cell exhibiting organelles condensed around the nucleus. (C) Scanning electron micrograph of a vesicle-bearing cell. Arrow indicates a piece of membrane fragment near the hole on the vesicle. (D) A freeze-fractured cell and vesicle.

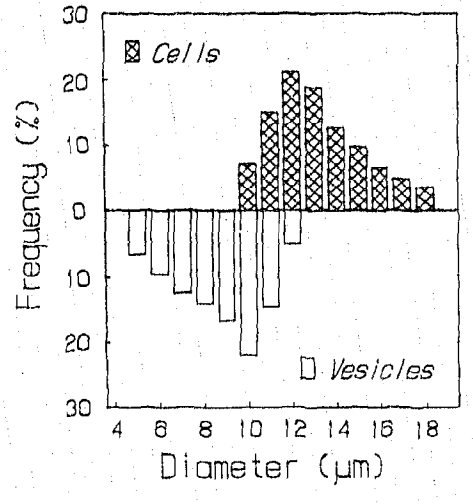

Fig. 3. Diameter distribution of untreated cells and vesicles purified by 3 min centrifugation at 60×g. The averaged diameter of vesicles is 8.7 μm.

firmed by a scanning micrograph (Fig. 2C). Since the vesicles were so large, they were too vulnerable to maintain a spherical shape during fixing and embedding as visualized by light microscopy. But they were seen as mostly spherical when using the advantages of freeze-fracture technique (Fig. 2D).

3.3. *Enzyme assay*

Since ouabain-sensitive Na^+,K^+-ATPase, NADH dehydrogenase, lactic dehydrogenase are marker enzymes of the plasma membrane, endoplasmic reticulum and cytosol, respectively, and the diameter measurement, and the vesicle and cell counting can be easily done under light microscope, we expressed the enzyme activity in a different manner from the conventional one which is based on protein content. This treatment would be expected to give a more direct comparison between the vesicles and cells. The results of enzyme analysis are given in Table I.

Table I

Comparison of marker enzyme activities of untreated cells and purified vesicles

	Ouabain-sensitive Na^+,K^+-ATPase[a]	NADH dehydrogenase[b]	Lactic dehydrogenase[c]
Cell	100	100	100
Vesicle	75.7	8.03	163.3

[a]Enzyme activity per unit surface area.
[b]Enzyme activity per unit internal volume.
[c]Enzyme activity per cell or vesicle.

4. DISCUSSION

The micrographs and the data of marker enzyme analysis suggest that the vesicles originated from plasma membrane. The exclusion of cellular organelles in the vesicles is clearly demonstrated by the asymmetric partitioning of organelles during vesiculation (Fig. 1A,B) and by the fact that the activity of lactic dehydrogenase is more than 1.5 fold higher in vesicles than in intact cells. From the data of lactic dehydrogenase, it can be estimated that one-third of the intracellular space is occupied by nucleus and organelles in intact cells. The remarking feature of the vesicles is that they are large enough to be visible under the light microscope. It would be expected that this unique characteristic would make the vesicles useful as a model membrane system in some situations where the application of membrane fragments or small vesicles is limited.

The rate of vesiculation of MA_{782} ascites cells induced by the electrolytes was concentration-dependent. Vesiculation was observed within 20 min after the addition of 3000 mM NaCl. Because more than 95% of the cells were vital as revealed by Trypan blue exclusion and were all subject to vesiculation, the phenomenon described in this report would not likely be the blebbing of moribund cells. Presumably, the vesiculation would be a result of salt precipitation of intracellular organelles. When cells are incubated in saline, electrolytes enter the cells via a passive process due to the huge concentration gradient and aggregation of organelles. As a result, considerable organelle-free space was produced within the cell's enclosure. In the meantime, some of the proteins in the plasma membrane will also be precipitated with the organelles 'anchoring' the membrane to the condensed organelles globe. Since the condensed organelle globe is much smaller in volume than intact cells, the spare membrane is used to form vesicles.

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