Secretion is a very important process occurring in all cells and is involved in the physiology of neurotransmission, and for the release of hormones and digestive enzymes. A number of diseases are known to result from defects in cell secretion. The area of cell secretion has been intensely investigated for over half a century. Until recently, it was commonly accepted that the final step in secretion is the total incorporation of secretory vesicle membrane into the cell plasma membrane leading to the release of intravesicular contents by diffusion, and the compensatory retrieval of excess membrane by endocytosis at a later time [1–10]. Studies within the past decade have finally revealed a completely different molecular mechanism of secretion and membrane fusion in cells [11–27]. Contrary to accepted belief, the mechanism of cell secretion is quite different, and a highly regulated process. These studies [11–17, 26] demonstrate that membrane-bound secretory vesicles dock and transiently fuse at the base of specialized plasma membrane structures called porosomes or fusion pores, to discharge vesicule contents. Contrary to what was previously suggested, there is no incorporation of the vesicle membrane at the cell plasma membrane. Recent studies further demonstrate that during secretion, secretory vesicles swell, enabling the discharge of intravesicular contents through porosomes [28–32]. These seminal findings [11–17, 26] have given rise to a new understanding of cell secretion.

**Abstract**

In the last decade, the discovery of a new cellular structure, the 'porosome' or fusion pore, and the discovery of SNARE-induced membrane fusion, and regulated expulsion of secretory products via secretory vesicle swelling, has finally provided us with an understanding of cell secretion at the molecular level. The current study was undertaken to further determine the structure of the porosome in resting pancreatic acinar cells and when co-isolated with zymogen granules, the secretory vesicles in exocrine pancreas in the rat. In agreement with earlier findings, our studies demonstrate the presence of porosomes at the apical plasma membrane where secretion occurs. Zymogen granules isolated from briefly stimulated pancreas using carbamylcholine, demonstrates as previously reported, the co-isolation of porosomes.

**Keywords:** porosome • fusion pore • secretion machinery • cell secretion

**Introduction**

Secretion is a very important process occurring in all cells and is involved in the physiology of neurotransmission, and for the release of hormones and digestive enzymes. A number of diseases are known to result from defects in cell secretion. The area of cell secretion has been intensely investigated for over half a century. Until recently, it was commonly accepted that the final step in secretion is the total incorporation of secretory vesicle membrane into the cell plasma membrane leading to the release of intravesicular contents by diffusion, and the compensatory retrieval of excess membrane by endocytosis at a later time [1–10]. Studies within the past decade have finally revealed a completely different molecular mechanism of secretion and membrane fusion in cells [11–27]. Contrary to accepted belief, the mechanism of cell secretion is quite different, and a highly regulated process. These studies [11–17, 26] demonstrate that membrane-bound secretory vesicles dock and transiently fuse at the base of specialized plasma membrane structures called porosomes or fusion pores, to discharge vesicule contents. Contrary to what was previously suggested, there is no incorporation of the vesicle membrane at the cell plasma membrane. Recent studies further demonstrate that during secretion, secretory vesicles swell, enabling the discharge of intravesicular contents through porosomes [28–32]. These seminal findings [11–17, 26] have given rise to a new understanding of cell secretion.

**Short Communication**

**Porosomes in exocrine pancreas: a study using electron microscopy**

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Received: January 10, 2006; Accepted: March 30, 2006

**Abstract**

In the last decade, the discovery of a new cellular structure, the 'porosome' or fusion pore, and the discovery of SNARE-induced membrane fusion, and regulated expulsion of secretory products via secretory vesicle swelling, has finally provided us with an understanding of cell secretion at the molecular level. The current study was undertaken to further determine the structure of the porosome in resting pancreatic acinar cells and when co-isolated with zymogen granules, the secretory vesicles in exocrine pancreas in the rat. In agreement with earlier findings, our studies demonstrate the presence of porosomes at the apical plasma membrane where secretion occurs. Zymogen granules isolated from briefly stimulated pancreas using carbamylcholine, demonstrates as previously reported, the co-isolation of porosomes.

**Keywords:** porosome • fusion pore • secretion machinery • cell secretion
secretion, and have been confirmed by other laboratories [33–38], including the present study.

Materials and methods

Isolation of rat pancreas for electron microscopy

Isolation and preparation of acinar cells for electron microscopy (EM) was performed by published procedures [11, 15, 16]. For each experiment, a rat weighing 75–120 g was sacrificed. The pancreas was immediately surgically removed and chopped into 0.5-mm³ pieces in ice-cold buffered saline, which were then fixed in 2.5% buffered paraformaldehyde for 30 min, and the pellets were embedded in Unicryl resin and were sectioned at 80-100 nm. Sections were transferred to coated specimen TEM grids, dried in the presence of uranyl acetate and methyl cellulose and examined in a transmission electron microscope (JEOL JEM 1010).

Isolation of zymogen granules from carbamylcholine-stimulated pancreas for electron microscopy

Chopped pancreas tissue was incubated for 5 min at 37°C in phosphate buffered saline containing 1 μM carbamylcholine. Zymogen granules were immediately isolated from the stimulated tissue, according to published procedures [11, 15, 16]. The pancreas from male Sprague–Dawley rats was dissected and diced into 0.5-mm³ pieces before being suspended in 15% (wt/vol) ice-cold homogenization buffer (0.3 M sucrose, 25 mM Hepes, pH 6.5, 1 mM benzamidine, 0.01% soybean trypsin inhibitor) and homogenized using 3 strokes of a Teflon glass homogenizer. The homogenate was centrifuged for 5 min at 300xg at 4°C. The supernatant fraction was then mixed with 2 vol of a Percoll–Sucrose–Hepes buffer (0.3 M sucrose, 25 mM Hepes, pH 6.5, 86% Percoll, 0.01% soybean trypsin inhibitor) and centrifuged for 30 min at 16,400xg at 4°C. Pure zymogen granules were obtained as a white pellet at the bottom of the centrifuge tube. The zymogen granules pellet was fixed in 2.5% buffered
paraformaldehyde for 10 min, placed in agar, the agar block formed after cooling was sliced, and embedded in Unicryl resin, followed by sectioning. Approximately, 80 nm sections were transferred to coated specimen TEM grids, dried in the presence of uranylacetate and methylcellulose and studied using the transmission electron microscope, both at high and low resolution.

Results and discussion

Our studies using resting pancreatic acinar cells, clearly demonstrate the presence of porosomes or fusion pores at the apical plasma membrane, as previously reported [11, 12, 15, 16]. Electron micrographs (Fig. 1, 2) show porosomes to be cup-shaped structures, which are roughly 90–120 nm in size. Fine fibrillar elements are also seen in the porosome in Fig. 1B. There are a number of fine cables or elements that appear to connect or anchor the porosome structure with the plasma membrane and most likely to the cytoskeletal network of the cell (Fig. 2). To be able to gain further understanding of the morphology of porosomes, pancreatic exocrine tissue was stimulated using 1 μM of the secretagogue carbacholamine, followed by isolation of zymogen granules, their mild fixation, and electron microscopy (Fig. 3). Again as previously reported [15], our study clearly demonstrates the co-isolation of porosomes with docked zymogen granules.

The presence of fusion pores had been suggested earlier, both from freeze-fracture electronmicroscopy [37] and electrophysiological studies on mast cells [38]. In both studies however, it was assumed that fusion pores are transiently formed as a result of plasma membrane invagination or indentation during cell secretion, and disappear following completion of the process. The pore formed either closed termed ‘flicker fusion’, or completely distended as a result of total incorporation of the vesicle membrane with the cell plasma membrane. The later model however, failed to account for the appearance of empty and partially empty vesicles following cell secretion. Further, in contrast to this model no change in vesicle number is demonstrated following secretion.

A breakthrough in our understanding of cell secretion came in the mid 90s, when atomic force microscopy on live pancreatic acinar cells demonstrated for the first time at nanometer resolution the presence of pores, 100–150 nm in diameter and 25–40 nm in depth at the apical plasma membrane, where secretory vesicles are known to dock and fuse to secrete digestive enzymes [11]. When the cell was stimulated to secrete, both the depth and opening of the pore increased by 35–50%, returning to resting size following completion of secretion [11]. Exposure of the cell to cytochalasin B resulted in a decrease in pore size and a significant loss (60–70%) in stimulable secretion. These studies suggested that the pore may be the fusion pore, where secretory vesicles fuse to extrude their contents from the cell. Further, it demonstrated that actin may be an important component of the pore structure-function and the pore to be a stable structural entity at the cell plasma membrane. The actual release of secretory products through the pore was subsequently demonstrated when immunoatomic force microscopy was performed on live pancreatic acinar cells [12]. An antibody against amylase (a major secretory enzyme within zymo-
gen granules) was gold conjugated, and specifically decorated the opening of the pore [12]. Subsequently, similar secretory pores have been identified in chromaffin cells [13], growth hormone cells of the pituitary gland [14], mast cells [26], the β cells of the endocrine pancreas [26], and in neurons [17]. The porosome has been isolated, its biochemical composition determined, and it has been functionally reconstituted into artificial lipid membrane [15, 16]. Including the present study, electron microscopy has confirmed the presence of poresomes in cells [16, 17]. The discovery of the porosome has finally helped unravel the molecular mechanism of cell secretion.

Acknowledgement

I thank my students and colleagues for critically reading the manuscript and for their helpful discussions and suggestions.

References


