Fusion pore or porosome: structure and dynamics

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Abstract
Electrophysiological measurements on live secretory cells almost a decade ago suggested the presence of fusion pores at the cell plasma membrane. Membrane-bound secretory vesicles were hypothesized to dock and fuse at these sites, to release their contents. Our studies using atomic force microscopy on live exocrine and neuroendocrine cells demonstrate the presence of such plasma membrane pores, revealing their morphology and dynamics at near nm resolution and in real time.

Introduction
Hormone release, neurotransmission, and enzyme secretion are fundamental physiological processes resulting from the fusion of membrane-bound secretory vesicles at the cell plasma membrane and consequent expulsion of vesicular contents. Membrane-bound secretory vesicles dock and fuse at specific plasma membrane locations following secretory stimulus. Earlier electrophysiological studies suggested the existence of ‘fusion pores’ at the cell plasma membrane. Following stimulation of secretion, the fusion pores at the plasma membrane become continuous with the secretory vesicle membrane (Monck et al. 1995). Using atomic force microscopy (AFM), the existence of the fusion pore was confirmed, and its structure and dynamics in both exocrine (Schneider et al. 1997, Cho et al. 2002d) and neuroendocrine (Cho et al. 2002b,e) cells determined at near nm resolution and in real time.

The resolving power of the light microscope is dependent on the wavelength of the light used and hence, at best, 300–400 nm objects can be observed. The recently discovered fusion pore in live secretory cells is cup-shaped, measuring 100–150 nm at its wide end and 15–30 nm in relative depth. As a result, it had evaded visual detection until recently (Schneider et al. 1997, Cho et al. 2002b,d,e). The development of the AFM (Binnig et al. 1986) has enabled the imaging of live biological samples in physiological buffers at near nm resolution. In AFM, a probe tip microfabricated from silicon or silicon nitride and mounted on a cantilever spring is used to scan the surface of the sample at a constant force (Albrecht et al. 1990). Either the probe or the sample can be precisely moved in a raster pattern using a xyz piezo tube to scan the surface of the sample (Fig. 1) (Binnig et al. 1986). The deflection of the cantilever measured optically is used to generate an isoforce relief of the sample (Alexander et al. 1989). Force is thus used to image surface profiles of objects by the AFM, allowing imaging of live cells and subcellular structures submerged in physiological buffer solutions. To image live cells, the scanning probe of the AFM operates in physiological or near physiological buffers, and may do so under two modes: contact or tapping. In the contact mode, the probe is in direct contact with the sample surface as it scans at a constant vertical force. Although high-resolution AFM images can be obtained in this mode of AFM operation, sample height information generated may not be accurate since the vertical scanning force may depress the soft cell. However, information on the viscoelastic properties of the cell and the spring constant of the cantilever, enables measurement of the cell height. In tapping mode, on the other hand, the cantilever resonates and the tip makes brief contacts with the sample, too brief to allow adhesive forces between probe tip and the sample surface. In the tapping mode in fluid, lateral forces are virtually negligible. It is therefore important that information on the topology of living cells be obtained using both contact and tapping modes of AFM operation. The scanning rate of the tip over the sample also plays a critical role on the quality of the image. Because cells are soft samples, a high scanning rate would influence the shape of the cell being imaged. Therefore, a slow movement of the tip over the cell would be ideal and results in minimal distortion and better resolved images. Rapid cellular events could, nonetheless, be monitored using section analysis obtained...
by a rapid line scan of the specific cell surface of interest. To examine isolated cells by the AFM, freshly cleaved mica coated with Cel-Tak have also been used with great success (Schneider et al. 1997, Cho et al. 2002b, d). The contents of the bathing medium as well as the cell surface to be scanned should be devoid of any large molecular weight proteins or cellular debris.

Cellular structures such as the fusion pore and the study of its dynamics could be examined using the AFM at nm resolution and in real time (Binnig et al. 1986, Alexander et al. 1989, Albrecht et al. 1990, Rugard & Hansma 1990, Schneider et al. 1997, Cho et al. 2002b, d, e). AFM-force spectroscopy allows imaging, at nm resolution and in real time, of objects such as live cells, subcellular structures or even single molecules submerged in physiological buffers. The discovery of this new cellular structure and its identification as the fusion pore, reveals a new understanding of the secretory process and, more importantly, into the workings of a living cell. In this article, the structure and dynamics of the fusion pore in live cells, examined using AFM, is described.

**New cellular structures**

Pancreatic acinar cells are polarized secretory cells. When stimulated with a secretagogue, membrane-bound secretory vesicles dock and fuse at the apical plasma membrane to release vesicular contents. Isolated live pancreatic acinar cells in physiological buffer, when imaged using the AFM (Schneider et al. 1997, Cho et al. 2002d), reveal at the apical plasma membrane highly stable and permanent circular ‘pits’ (Fig. 2a) measuring 0.4 µm – 1.2 µm in diameter, containing smaller cup-shaped ‘depressions’ within (Fig. 2b). Each depression measures around 100–150 nm in diameter (Fig. 2b), and typically 3–4 depressions are located within a pit. The basolateral membrane of acinar cells are, however, devoid of both pits and

**Figure 1** Schematic diagram depicting key components of an atomic force microscope.

**Figure 2** AFM micrographs revealing the structure of the fusion pore at the cell plasma membrane in exocrine and neuroendocrine cells. (a) A pit (white arrowheads) and four depressions (yellow arrowhead) are identified in a live pancreatic acinar cell. (b) Single fusion pore at high resolution. Scale bars = 100 nm.
depressions. High resolution AFM images of depressions in live cells further reveal a cone- or cup-shaped morphology (Fig. 2b). The depth of each depression cone measures approximately 15–30 nm. Similarly, both growth hormone (GH)-secreting cells of the pituitary gland and the chromaffin cell also possess pits and depression structures in their plasma membrane (Cho et al. 2002b, e), suggesting their universal presence in secretory cells.

**New structures are fusion pores/porosomes**

When exposed to a secretagog such as mastoparan, pancreatic acinar cells show a time-dependent increase (20–35%) in the diameter of depressions, followed by a return to resting size following completion of the process (Fig. 3). No demonstrable change in pit size is, however, detected during this time. Enlargement of depression diameter and an increase in its relative depth following exposure to secretagog, correlated with increased secretion. Exposure of pancreatic acinar cells to cytochalasin B, a fungal toxin that inhibits actin polymerization, results in a 15–20% decrease in depression size, and a consequent 50–60% loss in secretagog-induced secretion. Results from these studies suggested that depressions are the fusion pores or porosomes in pancreatic acinar cells. These studies further demonstrated the involvement of actin in the regulation of depression structure–function. Similar to the acinar cells, depressions in resting GH-secreting cells measure 154/±4·5 nm (mean ± s.e) in diameter. Exposure of the GH cell to a secretagog resulted in a 40% increase (215 ± 4·6 nm; P<0·01) in depression diameter, with no change in pit size.

Enlargement of depression diameter following exposure of acinar cells to a secretagog correlated with increased secretion. Additionally, actin depolymerizing agents known to inhibit secretion (Schneider et al. 1997) resulted in decreased depression size and accompanied loss in secretion. These studies further suggested that depressions were the fusion pores or porosomes. However, a more direct determination of the function of depressions was required. Hence, immuno AFM studies were performed. Using gold-conjugated antibody against a specific secreted protein, combined with AFM imaging, provided the means to determine if secretion occurs at depressions (Cho et al. 2002b, d). The membrane-bound secretory vesicles in

**Figure 3** Dynamics of depressions following stimulation of secretion. The top panel shows a number of depressions within a pit in a live pancreatic acinar cell. The scan line across three depressions in the top panel is represented graphically in the middle panel and defines the diameter and relative depth of the depressions; the middle depression is represented by red arrowheads. The bottom panel represents percentage of total cellular amylase release in the absence (W/O Addn.) or presence of the secretagog Mas 7 (20 μM). Notice an increase in the diameter and depth of depressions, correlating with an increase in total cellular amylase release at 5 min after stimulation of secretion. At 30 min after stimulation of secretion, there is a decrease in diameter and depth of depressions, with no further increase in amylase release over the 5 min time point. No significant increases in amylase secretion or depression diameter were observed in resting acini or those exposed to the non-stimulatory mastoparan analog Mas 17.
exocrine pancreas contain the starch digesting enzyme amylase. Using amylase-specific immunogold AFM studies, localization of amylase at ‘depressions’ following stimulation of secretion was demonstrated (Fig. 4a) (Cho et al. 2002d). These studies confirm ‘depressions’ to be the fusion pores or porosomes at the apical plasma membrane in pancreatic acinar cells, where membrane-bound secretory vesicles dock and fuse to release vesicular contents. Similarly, in somatotropes of the pituitary, gold-tagged GH-specific antibody was found to be selectively localized at depressions following stimulation of secretion (Cho et al. 2002b), again confirming depressions to be the fusion pores or porosomes.

Our studies on the role of actin in the regulation of depression structure and dynamics, clearly suggest actin to be a major component of the porosome complex. Target membrane proteins SNAP25 and syntaxin (t-SNARE) and secretory vesicle-associated membrane protein (v-SNARE), are part of a conserved protein complex involved in fusion of opposing bilayers (Rothman 1994, Weber et al. 1998). Since membrane-bound secretory vesicles dock and fuse at depressions to release vesicular contents, it is reasonable to suggest that plasma membrane-associated t-SNAREs are part of the fusion pore complex. In the last decade, a number of studies have demonstrated the involvement of cytoskeletal proteins in exocytosis, some directly interacting with SNAREs (Bennett 1990, Goodson et al. 1997, Prekereis & Terrian 1997, Schneider et al. 1997, Faigle et al. 2000, Miller & Sheetz 2000, Nakano et al. 2001, Ohyama et al. 2001). Actin and microtubule-based cytoskeleton have been implicated in intracellular vesicle traffic (Goodson et al. 1997). Fodrin, which was previously implicated in exocytosis (Bennett 1990), has recently been shown to directly interact with SNAREs (Nakano et al. 2001). Recent studies demonstrate that α-fodrin regulates exocytosis through its interaction with the syntaxin family of proteins (Nakano et al. 2001). The c-terminal coiled coil region of syntaxin interacts with α-fodrin, a major component of the submembranous cytoskeleton. Similarly, vimentin filaments interact with SNAP23/25 and control the availability of free SNAP23/25, for assembly of the SNARE complex (Faigle et al. 2000). Additionally, our recent studies confirm earlier findings and further demonstrate a direct interaction between actin and SNAREs (Jena et al. 2002). These findings suggest that vimentin, α-fodrin, actin and SNAREs may all be part of the fusion pore or porosome machinery. Purification and further biochemical characterization of the fusion pore is required to determine its composition. The possible involvement of proteins such as v-SNARE (VAMP or synaptobrevin), synaptoophysin and myosin, and their interaction when the fusion pore establishes continuity with the secretory vesicle membrane, should not be ruled out. The globular tail domain of myosin V is its binding site, and VAMP is bound to myosin V in a calcium independent manner (Ohyama et al. 2001). Further interaction of myosin V with syntaxin requires calcium and calmodulin. Studies suggest that VAMP acts as a myosin V receptor on secretory vesicles and regulates formation of the SNARE complex (Ohyama et al. 2001). Interaction of VAMP with synaptoophysine and myosin V has further been demonstrated (Prekereis & Terrian 1997, Miller & Sheetz 2000). Our recent studies reveal the composition of the fusion pore in pancreatic acinar cells. Further, structural details of the fusion pore have been confirmed using electron microscopy (Cho 1990).

![Figure 4](image_url)

**Figure 4** Fusion pores or porosomes dilate to allow expulsion of vesicular contents. (a and b) AFM micrographs and section analysis of a pit and two out of the four depressions or porosomes, showing enlargement of porosomes following stimulation of secretion. (c) Exposure of live cells to gold conjugated-amylase antibody (Ab) results in specific localization of gold to secretory sites. Note the localization of amylase-specific immunogold at the edge of depressions. (d) AFM micrograph of pits and depressions with immunogold localization is also demonstrated in cells immunolabeled and then fixed. Blue arrowheads point to immunogold clusters and the yellow arrowhead points to a depression or fusion pore.
et al. 2002c, Jena et al. 2002). The arrangement of t-/v-SNAREs resulting in fusion of opposing bilayers, and the presence of such a composition in the base of the fusion pore complex has been determined (Cho et al. 2002c). These studies provide a glimpse of the biochemical composition, structure, and regulation of the fusion pore or porosome (Fig. 5).

**Conclusion**

Fusion pores or porosomes in pancreatic acinar or GH-secreting cells are 100–150 nm wide cup-shaped structures at the plasma membrane. Membrane-bound secretory vesicles ranging in size from 0·2–1·2 µm in diameter dock and fuse at porosomes to release vesicular contents. Once secretory vesicles dock and fuse at porosomes, only a 20–35% increase in porosome diameter is demonstrated. It is therefore reasonable to conclude that secretory vesicles ‘transiently’ dock and fuse at depressions. In contrast to accepted belief, if secretory vesicle membranes were to completely incorporate and flatten out and become part of the plasma membrane at depressions, the fusion pore would distend much wider than that which is observed. Furthermore, if secretory vesicles were to completely fuse at the plasma membrane, there would be a loss in vesicle number following secretion. Examination of secretory vesicles within cells before and after secretion, demonstrates that the total number of secretory vesicles remains unchanged following secretion, however the number of empty and partially empty vesicles increases significantly, supporting the occurrence of transient fusion (Cho et al. 2002a). Earlier studies on mast cells also demonstrate an increase in the number of spent and partially spent vesicles following stimulation of secretion, without any demonstrable increase in cell size or change in vesicle number (Lawson et al. 1975). Other supporting evidence favoring transient fusion is the presence of neurotransmitter transporters at the synaptic vesicle membrane. These vesicle-associated transporters would be of little use if vesicles were to fuse completely at the plasma membrane, to be compensatory endocytosed at a later time. Although the fusion of secretory vesicles at the cell plasma membrane occurs transiently, complete membrane incorporation at the cell plasma membrane results when cells need to incorporate signaling molecules like receptors, second messengers or ion channels, at the plasma membrane.

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**References**


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