Scientifc report for the project:

Structural Analysis of O-glycosylated Domains During Mucin Supramolecular Assembly

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Abstract

Gel-forming mucin glycoproteins constitute the mucus that protects exposed epithelia in the body from physical and biological hazards. Mucins contain long segments, *i.e.*, thousands of amino acids, rich in proline, threonine, and serine (PTS). The threonine and serine side chains become heavily glycosylated during mucin bioassembly, and these post-translational modifications are essential for mucin function. In the major lung and intestinal mucins, the PTS regions are interrupted by multiple disulfide-rich domains of about 100 amino acids, called CysD domains. We aimed to gain insight into the function and organization of the PTS segments of mucins, as well as their relationship to CysD domains. To study mucin PTS regions, we took bottom-up and top-down approaches. In the bottom-up approach, we produced recombinant amino-terminal regions of different mucins containing different lengths and compositions of PTS segments and studied their modes of supramolecular assembly. In this research, we found that inclusion of long PTS segments (~400 amino acids) makes supramolecular assembly less robust *in vitro*, and more work is needed to determine whether it is possible to obtain information from such recombinant constructs. However, by examining different gel-forming mucins that naturally contain PTS regions of different lengths between their CysD domains, we are beginning to understand how co-evolution of PTS regions and CysD domains produces different types of mucin supramolecular assemblies, likely leading to different biophysical properties. In the top-down approach, we imaged mucin-containing secretory granules by transmission electron microscopy (TEM) and scanning transmission electron microscopy after focused ion beam (FIB) milling of detached colon epithelial cells. In contrast to similar, control experiments done on endothelial cells, which confirmed that we could detect the organization of von Willebrand factor tubules in Weibel Palade bodies, no underlying order was seen in mucus vesicles in lamellae prepared by FIB milling. However, we are still working towards imaging large and mature colon goblet-cell granules (~10 μm diameter) in intact colon epithelium, for which technical challenges in freezing of tissues for subsequent FIB milling must be overcome.

Objectives

The overall objective of the project was to determine whether there is structural order to glycosylated PTS regions in mucins and how these regions affect mucin bioassembly in cells prior to secretion. The two specific aims were 1) to determine whether and how long PTS segments influence the *in vitro* supramolecular assembly of the amino-terminal regions of mucins and 2) whether order (on the scale of tens of nanometers) can be detected in mucin-containing vesicles and granules in goblet cells. For the first aim, we prepared recombinant segments of multiple mucins, beginning at the amino termini and extending through one or more PTS regions and CysD domains. We then examined the ability of these fragments to undergo supramolecular assembly when placed in the pH and salt conditions representative of the Golgi apparatus and mucin granules in cells. For the second aim, we isolated epithelial cells from colon tissue, allowed them to adhere to electron microscopy grids, cryo-preserved the cells, and then performed FIB milling of the frozen cells to generate thin (200-300 nm) lamellae in regions of mucin-containing vesicles. Lamellae were then imaged by TEM.