Molecular structure, packing and release of MUC2 with relevance to Cystic Fibrosis

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Introduction: Cystic fibrosis (CF) is the most common autosomal recessive lethal disease among Caucasians, where it affects about 1 in 2500 live birth [1]. CF affects fluid transporting tissues, where extremely viscous mucus is built up, especially in the airways, pancreas and intestine. CF is caused by different mutations in the CFTR gene coding for the cAMP regulated chloride channel, the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR functions as a chloride channel but it also transports bicarbonate [2].

O-glycosylation makes up the core of the mucus gel, which covers all mucosal surface of the body and plays a central role in protection and hydration [3]. Typical for the gel forming mucins is a large size and the capability to form multimers. MUC2 dimerizes in the endoplasmic reticulum via its C-terminus, becomes heavily O-glycosylated in the Golgi and polymerizes via the N-terminus by disulfide-bonded trimerization [4]. After multimerization, MUC2 is stored in the goblet cells in secretory granules in a high [Ca²⁺] and low pH environment. It is suggested that the N-terminus controls this packing [5]. During secretion, by not fully understood mechanisms, the protein encounters the extracellular milieu of low [Ca²⁺] and high pH, which seem to make unpacking and release possible [5]. The aim of this study is to elucidate organization and structure of MUC2 N-terminus when it is packed and secreted.

Methods: The N-terminal part of MUC2 was expressed in CHO cells (figure 1, model). The secreted recombinant mucin including von Willebrand D1-D2-D’D3 (VWD) domains and a CysD domain was purified from culture medium by anion exchange and gel filtration chromatography and analyzed by density gradient centrifugation, transmission electron microscopy (TEM) and immuno gold labeling. The pH in the buffers was varied in the range from 5.2 to 8 with or without calcium to mimic the conditions of the secretory pathway and extracellular environment. Samples were adsorbed onto carbon coated EM grids and negatively stained at room temperature or plunge frozen in liquid ethane (-190°C). Determination of the location of the core domain (D’D3) of the N-terminal construct of MUC2 was performed by using a gold labeled antibody specific for VWD3. Single particle reconstructions were performed using the EMAN1 software. Top views of strictly symmetric particles (i.e. 5- or 6-sided polygons) in different orientations were selected for 2D reconstruction using the 2D-refine program.

Results: When pH was low, pH 6.2, and calcium present, 5-, 6- and 7-sided regular polygons were observed in TEM. Without calcium these vanished with increasing pH. The best condition for polygon formation was at pH 6.2 with calcium. 2D refinements of the projections showed rotational 5- and 6- fold symmetry (figure 2), where the inner cores encompass circles with a diameter of 13 and 19 nm, respectively. The length of the sides was the same independently of the polygon size, ~ 19 nm. Thus the polygons might be assembled from similar repeating units, i.e. N-terminal units with the D’D3 and CysD domains located in the vertices, which has been shown by immuno gold labeling [5]. When the specimens were subjected to 100 mM NaHCO₃, the polygons effectively started to ‘dissolve’ (Figure 3). Isolation of MUC2-N rings by density gradient centrifugation showed large complexes of MUC2-N concatenated rings (figure 4) that also were effectively dissolved, when NaHCO₃ was added [5].

Conclusion and relevance: The formation of N-terminal polygons is probably vital for proper packing and release of full length MUC2 and indicates a pH- and Ca²⁺-dependent supramolecular
organization of the mucin. Bicarbonate likely has an important function in removing and stabilizing calcium ions from the densely packed mucins. Dysregulation of this process, also resulting in low surface pH, seems to harmfully affect the possibility for the mucus to expand properly when secreted and could explain the extreme viscosity of the produced mucus seen in CF [2]. Understanding the mechanisms of mucin packing and secretion will assist in designing novel therapeutics.

References

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Figure 1. MUC2 with the recombinant N-terminal protein, D1D2D’D3, and the CysD domain encircled

Figure 2. Averaged projection structures of 5- and 6-sided negatively stained polygons built up of the same structural elements, bar 20 nm

Figure 3. Polygons effectively started to ‘dissolve’ when exposed to bicarbonate (right), control (left), bar 100 nm

Figure 4. MUC2 N-terminal sheet (concatenated polygons) in negative stain, bar 100 nm