Granule formation in pancreatic acinar cells

The pancreas is an important gland organ within the digestive and endocrine system of vertebrates. It is both an endocrine and exocrine gland. The endocrine part produces several important hormones such as insulin which are released in the bloodstream. The exocrine part is responsible for the production and secretion of pancreatic juice which contains digestive enzymes that pass to the small intestine. The acinar cells of the exocrine pancreas are specialised in the synthesis, sorting, storage and regulated secretion of the complex mixture of digestive enzymes, which are packaged in a condensed and inactive form into zymogen granules. These large and abundant membrane-bound secretory compartments are stored underneath the apical membrane of the acinar cells and release their cargo upon neuronal or hormonal stimulation by fusion with the apical plasma membrane. The secreted digestive enzymes reach the small intestine via the pancreatic duct system, where they are activated through proteolytic cleavage. Premature activation of the digestive enzymes within the acinar cells can however be devastating and result in cell and tissue damage, severe inflammation (pancreatitis) and death. Despite its medical and biological importance, the molecular mechanisms of zymogen granule formation and sorting of digestive enzymes are still incompletely understood. This also applies to the regulation and maintenance of the size and the shape of the relatively large zymogen granules (up to 1 µm in diameter). Highly sulphated and glycosylated proteins, so called proteoglycans, have been proposed to support zymogen granule formation and to concentrate at the granule membrane, but their role in granule formation remains unclear.

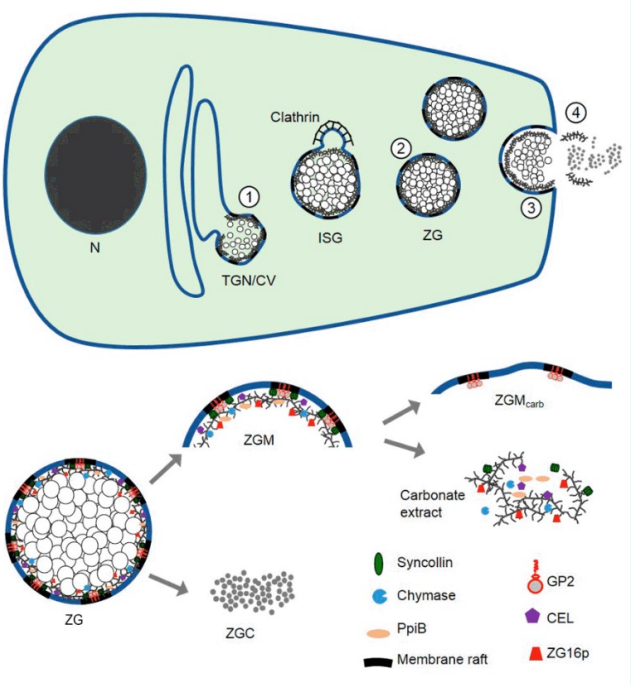

Fig. 1.

By exploiting a proteoglycan-specific dye (Cupromeronic Blue) we succeeded in localising proteoglycans within zymogen granules at the ultrastructural level. Proteoglycans were mainly present attached to the inner side of the granule membrane, where they are supposed to contribute to the formation of a submembranous protein scaffold (“submembranous matrix”). Biochemical analysis of granule subfractions confirmed that proteoglycans were mainly present in the membrane fraction and could be released from the membrane by chemical treatment. Interestingly, treated membranes were no longer curved but had a linear appearance indicating that proteoglycans support membrane shape and curvature of the relatively large granules. To investigate this further, we treated AR42J cells, a pancreatic cell model, with a pharmacological inhibitor of proteoglycan synthesis. Treatment perturbed normal granule formation and ultrastructural studies revealed that the granules formed were smaller in diameter and of irregular shape. An altered granule size was confirmed by centrifugation and separation of granules of controls and treated cells in density gradients. We also assessed the secretion of digestive enzymes after inhibition of proteoglycan synthesis and found that the granule cargo was released in an unstimulated way. This further suggests that after inhibitor treatment part of the digestive enzymes is not located in typical, stimulus-sensitive storage granules and is misrouted to the constitutive secretory pathway.

We propose that proteoglycans within zymogen granules are important accessory components in granule biogenesis which can support multiple steps in granule formation: proteoglycans can interact electrostatically and through specific protein-protein and carbohydrate-protein binding domains with the secretory proteins of the granule content. In addition to a potential mechanical function in granule formation and in the maintenance of granule shape and stability, they contribute to the proper packaging and sorting/membrane binding of cargo (Fig. 1). Additional functions may be the modulation of enzyme function before or after secretion and granule fusion/cargo release. It will be a challenge for future studies to identify and characterize these proteoglycans on the molecular level, and to elucidate their complex functions and interactions in health and disease.

Publication

Proteoglycans support proper granule formation in pancreatic acinar cells.
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