

## Biosynthesis and Sorting of Myeloperoxidase in Hematopoietic Cells

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**SUMMARY:** The neutrophil granulocytes have a critical role in innate immunity through killing of phagocytized microorganisms, in which myeloperoxidase (MPO) participates. MPO is stored in cytoplasmic azurophil lysosome-like granules together with other antibiotic proteins and digestive enzymes. During passage in the secretory pathway pro-MPO is folded, subjected to oligosaccharide modification, and retrieval from constitutive secretion to become targeted to azurophil granules for final processing and storage. Propeptide-deleted MPO precursor was found not to be processed to mature MPO and not to be targeted for storage but instead degraded or secreted. This indicated that the propeptide of the MPO precursor was a prerequisite for the final processing and granule targeting of proMPO. When the MPO propeptide was expressed as a chimera with a normally secretory protein, the ER retention of the chimera was prolonged compared with that of the native protein. Thus, the propeptide of MPO precursor may also mediate the normally long ER-residence of proMPO. Both mature MPO and secreted proMPO contained complex oligosaccharide side chains indicating that proMPO and, thus, mature MPO has passed the medial Golgi stack where complex oligosaccharides are formed, and exited at TGN like other proteins targeted for azurophil granules.

MPO is a constituent of both neutrophils and monocytes. It was discovered in 1941 by Agner who isolated the enzyme from canine pus, and designated it verdoperoxidase since it gives neutrophils and pus their green color. Some CD34<sup>+</sup> hematopoietic progenitor cells in the bone marrow also express MPO, although this protein starts to accumulate during the promyelocyte stage of differentiation after which the synthesis decreases during terminal maturation. Hematopoietic cells such as neutrophils, mastcells, CTLs and NK-cells contain secretory lysosomes that combine three major functions: storage, regulated secretion and lysosomal activity. The secretory lysosomes of neutrophils, designated azurophil granules, store MPO and other antimicrobial proteins.

MPO is the major protein of azurophil granules of neutrophils, and its biosynthesis and processing have been characterized in the past (1-3). For a complete understanding several issues remain however to be explored: the very long residence time of proMPO in the ER, the exit site for proMPO in the Golgi complex, the role of the propeptide for targeting, the final processing in granules, and the explanation of constitutive selective secretion of proMPO. Mature MPO is a homodimer with a light and a heavy subunit, connected by a disulfide bond between the two heavy subunits. A

heme group is covalently bound to each heavy subunit, but may interact with both subunits in the intact protein (3). The MPO gene is translated as a single peptide precursor with an amino-terminal signal peptide, followed downstream by the propeptide, the light and the heavy subunit (4). During their passage in the secretory pathway proteins are folded, oligosaccharides modified, and granule proteins retrieved from constitutive secretion to become sorted for storage (Fig. 1). ApoproMPO receives one high mannose oligosaccharide on the propeptide and four on the heavy subunit (2). Some high mannose side chains become phosphorylated in cis-Golgi cisternae. Calreticulin (CRT) and calnexin (CLN), molecular chaperones of the ER, facilitate folding of apoproMPO in the ER and CRT is suggested to facilitate the insertion of a prosthetic heme group (5). The heme insertion generates an enzymatically active precursor, the proMPO.

We have studied the processing and sorting of MPO and other secretory lysosome proteins. In order to do that, we have transfected cDNA to murine myeloid cell lines and transduced normal hematopoietic progenitor cells retrovirally, to constitutively express the protein. Propeptide-deleted MPO precursor was found not to be processed to mature MPO and not to be targeted for storage but instead degraded or secreted, indicating that the propeptide of the MPO precursor was a prerequisite for the final processing and

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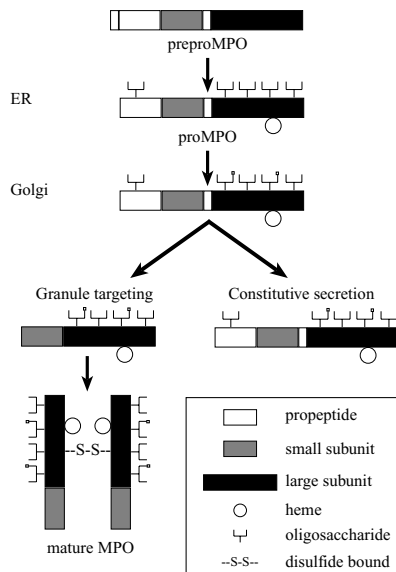


Fig. 1. Biosynthesis and processing of MPO.

targeting of proMPO to granules (6) (Fig. 2). The propeptide-deleted MPO was, however, still secreted (6) indicating that undegraded propeptide-deleted MPO can be exported from ER to Golgi but not sorted for storage. Propeptide deletion resulted also in abolished late processing of the MPO precursor (6), indicating that the propeptide is necessary for final processing of MPO, that accordingly occurs in the post-Golgi pregranule/granule compartment.

The proMPO processing is very slow because of an unusually long residence time in the ER before export. We observed that the addition of the MPO propeptide to other proteins to produce chimeras prolonged their retention time too in the ER. Thus, chimeras that contained the MPO propeptide showed prolonged ER-retention compared to the native protein without the MPO propeptide. The prolonged retention was independent of the chaperones calreticulin and calnexin, normally involved in ER quality control (7). The propeptide of proMPO may therefore be responsible for the long ER residence time normally observed for MPO precursor perhaps to provide enough time for heme incorporation.

The MPO propeptide might act as sorting signal. We asked whether the propeptide could target a normally secretory protein for granule storage. Alpha-1 microglobulin was chosen for this purpose normally being a secretory liver protein. A chimera was generated between the MPO propeptide and alpha-1 microglobulin (7). The chimera was exported from ER suggesting normal folding. And, it was targeted for granules. On the road, the propeptide was eliminated as well, and some chimera was constitutively secreted. The result seemed to support the hypothesis, but the control experiment showed that alpha-1 microglobulin alone was also targeted for granules without a need for the MPO-propeptide. We realized that targeting in hematopoietic cells might not be protein-specific. Non-hematopoietic proteins as well could be sorted for storage in a cell-specific manner when expressed in these cells. We have taken advantage of this observation in other research that suggests a potential for using the storage organelles of hematopoietic cells as vehicles for targeting sites of inflammation with therapeutically active agents. The neutrophil MPO-positive granules would be suit-

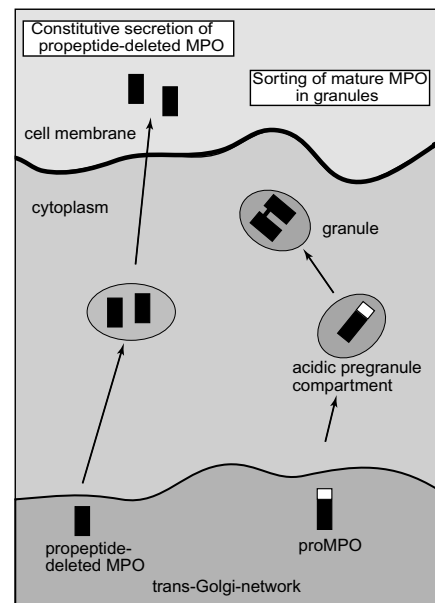


Fig. 2. Intracellular trafficking of MPO and propeptide-deleted MPO.

able vehicles for delivering proteins into the phagosome to promote antimicrobial defence.

In conclusion, elimination of the propeptide prevented MPO targeting and blocked maturation indicating that the propeptide is a critical element in sorting and processing of MPO. In addition, our data suggested that the propeptide might be responsible for the long ER residence time for the MPO precursor that may be needed for heme incorporation.

#### ACKNOWLEDGMENTS

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