The Role of the Propeptide for Processing and Sorting of Human Myeloperoxidase*

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Myeloperoxidase (MPO), stored in azurophil granules of neutrophils, is critical for an optimal oxygen-dependent microbicidal activity of these cells. Pro-MPO goes through a stepwise proteolytic trimming with elimination of an amino-terminal propeptide to yield one heavy and one light polypeptide chain. The propeptide of MPO may have a role in retention and folding of the nascent protein into its tertiary structure or in targeting of pro-MPO for processing and storage in granules. A propeptide-deleted pro-MPO mutant (MPOΔpro) was constructed to determine if deletion of the propeptide interferes with processing and targeting after transfection to the myeloid 32D cell line. Transfection of full-length cDNA for human MPO results in normal processing and targeting of MPO to cytoplasmic dense organelles. Although the efficiency of incorporation was lower for MPOΔpro, both pro-MPO and MPOΔpro showed heme incorporation indicating that the propeptide is not critical for this process. Deletion of the propeptide results in synthesis of a protein that lacks processing into mature two-chain forms but rather is degraded intracellularly or secreted. The finding of continued degradation of MPOΔpro in the presence of lysosomal agents or brefeldin A rules out that the observed degradation takes place after transfer to granules. Intracellular pro-MPO has high mannose oligosaccharide side chains, whereas stored mature MPO was found to have both high mannose and complex oligosaccharide side chains as judged by only partial sensitivity to endoglycosidase H. The propeptide may normally interfere with the generation of certain complex oligosaccharide chain(s) supported by the finding of high mannose side chains in secreted pro-MPO and lack of them in MPOΔpro that contained complex oligosaccharide side chains only. In conclusion, elimination of the propeptide of pro-MPO blocks the maturation process and abolishes accumulation of the final product in granules suggesting a critical role of the propeptide for late processing of pro-MPO and targeting for storage in granules.

Neutrophil granulocytes are specialized for a role in host defense. A regulated pathway targets enzymes and antibiotic proteins to a storage compartment in these cells consisting of cytoplasmic azurophil, specific, and gelatinase granules formed sequentially, whereas a constitutive pathway exports proteins to the cell surface (1). Azurophil granules are thought to be specialized lysosomes, and their protein constituents are often subject to posttranslational glycosylation and proteolytic trimming similar to that of lysosomal enzymes (2). A retention mechanism may be necessary to avoid constitutive secretion of granule proteins, and a condensation mechanism is necessary for efficient packaging. Signals for targeting storage in granules have been sought within the structure of neutrophil granule proteins. For instance, a pro-region segment is necessary for targeting to granules of neutrophil defensins (3), but carboxy-terminal prodomains or asparagine-linked carbohydrates of hematopoietic serine proteases are not required in targeting storage in granules (4, 5). Myeloperoxidase (MPO)† of azurophil granules plays a major role in the oxygen-dependent killing of microorganisms after release into phagolysosomes by amplifying the effects of oxygen derivatives formed during the respiratory burst (6). Pro-MPO undergoes extensive processing, including the removal of an amino-terminal propeptide not found in mature MPO. Therefore, in this work we have investigated whether the propeptide of MPO has a role in intracellular trafficking and targeting to granules.

The processing steps for MPO are shown in Fig. 1. Mature MPO is a 150-kDa tetramer composed of two glycosylated 59–64-kDa heavy subunits and two unglycosylated 14-kDa light subunits as a pair of protomers linked together by a disulfide bond (7). Each heavy subunit carries a covalently bound heme prosthetic group (8), although the crystal structure of canine MPO suggests that heme of the intact molecule associates with both subunits (9). The primary translation product undergoes cotranslational glycosylation with production of 89-kDa heme-free apoprotein MPO followed by incorporation of heme and conversion into enzymatically active pro-MPO (7). Processing and maturation of pro-MPO is a slow process (10) that can be accomplished only after acquisition of heme (11–13). Calreticulin, a calcium-binding protein that resides in the ER, has been suggested to function as a molecular chaperone and facilitate the critical folding of apoprotein MPO to allow insertion of heme followed by conversion to pro-MPO (14). The stepwise processing of pro-MPO has been investigated in myeloid cells (10, 15–20), and the results obtained are consistent with those earlier deduced from cDNA sequence data. Thus, during subsequent processing of pro-MPO the amino-terminal propeptide, a small peptide between the light and heavy chains, and a single serine residue at the carboxyl-terminal are removed (21). Intermedi-

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‡ The abbreviations used are: MPO, myeloperoxidase; [4-14C]ALA, δ-[4-14C]aminolevulinic acid hydrochloride; ER, endoplasmic reticulum; Endo-H, endoglycosidase H; N-glycanase, N-glycosidase F; PCR, polymerase chain reaction; BFA, brefeldin A.
EXPERIMENTAL PROCEDURES

Materials—The eucaryotic expression vector pCDNA 3 was from Invitrogen, British Biotechnology, Oxon, UK. The vector provides a cassette for MPO in retention and folding of the nascent protein into its mature form. To obtain a full-length clone for transfection studies, an approximate 500-base pair fragment, originating from the 5' end of the mRNA, was isolated by PCR amplification. This fragment was used to transfect the murine myeloid 32D clone cell line.

FIG. 1. The processing steps of MPO. The primary translation product undergoes cotranslational cleavage of the signal peptide followed by N-linked glycosylation to generate apopro-MPO. Initial processing also includes acquisition of heme, which yields enzymatically active pro-MPO. The stepwise processing into mature dimeric MPO includes removal of the propeptide. The amino-terminal propeptide has been observed with molecular masses of 81 and 74 kDa (10, 18, 19, 22) of which the smaller can be converted directly into mature MPO after cleavage between the heavy and the light subunit (19, 22). This finding suggests that the amino-terminal propeptide, which does not seem to be part of the 74-kDa form, is removed during an intermediate step before final processing.

One can envision a role for the amino-terminal propeptide of MPO in retention and folding of the nascent protein into its tertiary structure or in targeting pro-MPO to pregranule structures for further processing and storage in granules. A propeptide-deleted pro-MPO mutant (MPO(pro)) was constructed to determine if propeptide deletion interferes with processing and targeting. In this work, we describe the consequences of these manipulations for posttranslational processing, intracellular sorting, and constitutive secretion after transfection of the cDNA for MPO and MPO(pro) into the murine myeloid 32D clone cell line.

Myeloperoxidase Processing

FIG. 2. Schematic view of MPO and of the MPO deletion mutant (MPO(pro)). PCR primers used for construction as described under “Experimental Procedures” are indicated with arrows. Amino acids are indicated by three-letter symbols. SP, signal peptide; PRO, propeptide; LIGHT, light chain of mature MPO; HEAVY, heavy chain of mature MPO.

pMP503, ATCC 57894). This clone lacks the coding region for the amino-terminal part of the protein. To obtain a full-length clone for transfection studies, an approximate 500-base pair fragment, originating from the 5' end of the mRNA, was isolated by PCR amplification. This fragment was cloned as an RNAseqI informative fragment, and the entire nucleotide sequence was determined for two separate clones. One of the clones was found to have an identical sequence to that for MPO. To obtain the full-length MPO clone, two separate fragments were ligated into the vector pcDNAIneo, one XbaI EcoRI fragment originating from the pMP503 clone and one HindIII XbaI fragment from the PCR clone. After sequence analysis of 5' and 3' ends of the resulting clone and restriction mapping for a panel of internal sites, this clone was used for the subsequent transfection studies and as a starting material for the construction of the MPO(pro).

Construction of cDNA of MPO Lacking the Propeptide (MPO(pro))—For site-directed mutagenesis cDNA of human MPO (pcDNAIneoMPO) was used as template in a two-step “spliced overhang extension” polymerase chain reaction in the following way. In the first reaction two separate amplifications with 100 ng of DNA template in a 20-cycle PCR produced two fragments of myeloperoxidase positional amino-terminally and carboxytermically of the propeptide (Pro46-Gly80), respectively (Fig. 2). By design of the primers, the “Kozak” consensus leader sequence for maximum translational efficiency was introduced 5' to the ATG initiation codon, and the flanking restriction enzyme sites HindIII and BamHI were included for subsequent cloning into plasmid. The PCR primers in the two amplifications were upstream 5'-GACTTCCAAGCGTACCAGTGCGGTGCCCTGGCTGTGGGCG-3' (primer 1) plus downstream 5'-CGGGCGATCTCCACCCACGTC-GGCTGCGGGTGGCCGAATGAG-3' (primer 2) and upstream 5'-GACGTGGGTTGCTAGTGGCCGTCGGCCCGG-3' (primer 3) plus downstream 5'-CTTGAGGGAT-CTAGGGAGCTTCCACTCAGAAG-3' (primer 4), respectively (start and stop codons in boldface and restriction enzyme sites underlined). The PCR products were isolated on agarose gel, mixed, and subjected to a second 20-cycle splicing PCR amplification with primers 1 and 4, thus creating MPO lacking the propeptide (MPO(pro)). The resulting PCR product was digested by HindIII and BamHI, followed by isolation on agarose gel and cloning into plasmid (pcDNA3) to create the expression vector pcDNA3/MPO(pro).

All PCRs were performed in a Perkin-Elmer 480 Thermal Cycler using Pfu polymerase (Stratagene, La Jolla, CA) according to the manufacturer’s instructions.

Cell Culture—32D clone 3 cells (23, 24), kindly provided by G. Rovera (Philadelphia, PA) were grown in complete medium consisting of Iscove’s modified Dulbecco’s medium supplemented with 10% heat-inactivated fetal bovine serum and 30% WEHI-conditioned medium as a source of interleukin 3 (25). The cell cultures were kept in 5% CO2 at 37 °C in a fully humidified atmosphere. Exponentially growing cells were used in all experiments.

Transfection Procedure—32D cells were transfected with pcDNAIneo/MPO and pcDNA3/MPO(pro) using the Bio-Rad Electroporation Apparatus (Bio-Rad) with electrical settings of 960 microfarads and 300 V as described previously (5). Forty-eight hours after electo-
poration, geneticin (1 mg/ml) was added to select for recombinant clones expressing the geneticin resistance of pcDNA3. Individual clones growing in the presence of antibiotic were isolated, expanded into mass cultures, and screened by biosynthetic radiolabeling for expression of the protein encoded by the transfected cDNA. Clones with the most pronounced expression were chosen for further experiments.

Biochemical Procedures—Biochemical radiolabeling of newly synthesized proteins was performed as described (26). Briefly, cells were starved for 30 min in methionine/cysteine-free medium, followed by radiolabeling with 15 or 30 µCi/ml [35S]methionine/[35S]cysteine for 30 or 60 min. In experiments with [4-14C]ALA labeling, cells were incubated with 25 µCi/ml for 3 h for radiolabeling. In chase experiments, following radiolabeling, cells were resuspended in complete medium. At timed intervals, cells were withdrawn and lysed or homogenized for subcellular fractionation.

Subcellular Fractionation—Subcellular fractionation was performed as described (26). Briefly, the postnuclear cell homogenate was fractionated in a Percoll density gradient, after which nine fractions were solubilized, and biosynthetically labeled MPO or MPO-pro was precipitated with polyclonal anti-MPO (29) and subjected to electrophoretic analysis followed by fluorography as described previously (26, 30).

Digestion with Endo-H and N-Glycanase—The susceptibility of MPO and MPO-pro to digestion with Endo-H and N-glycanase was determined as described (26, 30).

RESULTS

Construction of Full-length and Mutated MPO and Establishment of Stable Transfectants—To determine whether the propeptide of pro-MPO carries a targeting signal for granules, a mutant form of MPO lacking the propeptide (MPO-pro) was constructed by polymerase chain reactions as described under “Experimental Procedures.” The sequence encoding 122 amino acids from pro53 to Gly164 (numbered from the methionine constituting the translation initiation site) was deleted from MPO cDNA leaving the first three residues of the propeptide and the entire signal peptide intact (Fig. 2). If the propeptide plays a role for sorting, MPO-pro protein would, unlike intact pro-MPO, not be targeted to granules. Likewise, if the propeptide plays a role for folding of nascent protein, the mutant protein might be misfolded and retained in the ER.

Wild type MPO and MPO-pro were transfected to 32D cells, and stable cell clones were established. Clones with synthesis of protein from transfected cDNA were chosen for further experiments in which the consequences of the MPO mutation were investigated. The murine origin of the cell line facilitates the detection of expression of transfected human proteins by biosynthetic radiolabeling followed by immunoprecipitation. No endogenous synthesis of MPO in 32D cells is detected with the antiserum used (data not shown). 32D cells have cytoplasmic granule-like vacuoles that have been shown to be able to accumulate human neutrophil granule constituents such as defensins expressed from DNA transfected into these cells (9). In addition, the human hematopoietic serine proteases cathepsin G and proteinase 3 have been successfully transfected to 32D cells and targeted to the granule-like vacuoles (5, 32).

These experiments were also found to have the machinery for processing of MPO (see below).

Human Wild Type MPO in 32D Cells Is Processed and Targeted to Granules—Stable 32D cells transfected with wild type cDNA of human MPO show a biosynthesis and processing pattern of MPO similar to that of human myeloid cells expressing MPO. The initially detectable protein is a proform of molecular mass 89 kDa (pro-MPO) (Fig. 3). As observed earlier in promyelocytic HL-60 (9, 22, 33) and in PLB 985 cells (11), processing of pro-MPO into the mature form is slow. A slow processing of pro-MPO is also observed in transfected 32D cells. Therefore, a 64-kDa heavy chain and a 15-kDa light chain, representing mature MPO, begin to occur between 6 and 24 h of chase of the radiolabel (Fig. 3). Additional MPO species with molecular masses of approximately 45 kDa, precipitated with anti-MPO, increase with chase of the radiolabel. These peptides are known to be the result of autolytic cleavage of the heavy subunit (34). Similar to the behavior of endogenous MPO in myeloid cells, constitutive secretion of pro-MPO to medium proceeds continually from 32D cells during chase of the radiolabel (Fig. 3).

MPO is normally targeted to azurophil granules of the neutrophil series for storage. To investigate targeting in MPO-transfected 32D cells, pulse-chase radiolabeling experiments followed by subcellular fractionation were performed (Fig. 4).

Radiolabeled MPO was found to be slowly translocated to dense fractions containing the granule-like vacuoles, where it is clearly visible after 22 h of chase. Translocated (granule-associated) MPO is present almost exclusively in its mature form consisting of a 64-kDa heavy and 15-kDa light chain. These results are consistent with earlier data from investigations on the processing of endogenous MPO in myeloid cells (10, 15–20) and demonstrate that 32D cells can process human pro-MPO into mature MPO that is at least partially targeted to a dense subcellular fraction containing granules.

Both Wild Type MPO and Propeptide-deleted MPO (MPO-pro) Incorporate Heme—Proteolytic processing of endogenous wild type MPO precursor to the mature storage form in granules requires incorporation of heme into pro-MPO (11–13). Thus, heme incorporation occurs prior to removal of the propeptide, and it is therefore of interest to determine whether the propeptide is necessary for incorporation of heme. Wild type MPO and propeptide-deleted MPO (MPO-pro) in infected 32D cells were therefore compared in this respect. Cells were radiolabeled with [4-14C]ALA, a precursor of heme synthesis, followed by immunoprecipitation with an anti-MPO antibody. As expected, the proform of wild type MPO incorporates heme, indicated by labeling of the protein with [4-14C]ALA (Fig. 5), confirming earlier results (9). Incorporation of heme into the propeptide-deleted MPO (MPO-pro) is also seen...
onine/[35S]cysteine for 30 min followed by chase of the label for 5 and 22 h. At these time points, 100 × 10⁶ cells were homogenized after which subcellular fractionation of the postnuclear supernatant was performed by centrifugation in Percoll followed by collection of nine subcellular fractions, fraction 9 containing all the cytosol. The fractions were lysed and subjected to immunoprecipitation with anti-MPO. Immunoprecipitates were analyzed as described in the legend to Fig. 3. The fluorogram was exposed for 11 days. The positions of the pro-MPO (pro), the heavy (α), and the light (β) subunits are indicated to the right with arrows. Peak activities of β-hexosaminidase, fraction 2, and galactosyltransferase, fraction 6, indicate the position of lysosomes and Golgi elements, respectively. (Fig. 5), and this form has a molecular mass of 76 kDa. Thus, the presence of the propeptide is not necessary for incorporation of heme into the proform of MPO. On the other hand, the relative efficiency of insertion of heme seemed to be lower for MPOΔpro compared with the normal proform. This comparison was possible to make as control radiolabeling with [35S]methionine/[35S]cysteine showed immunoprecipitates with similar density for both MPO and MPOΔpro also when visualized through shorter exposure time of the fluorogram than in Fig. 5 (not shown).

**Lack of Processing and Targeting to Granules of Propeptide-deleted MPO**—32D cells expressing MPOΔpro show an abnormal biosynthesis and processing pattern of MPO (Fig. 6). As expected, a 76-kDa polypeptide is synthesized that may correspond to the size of a pro-MPO that lacks propeptide. However, deletion of the propeptide results in synthesis of a protein that lacks processing into mature two-chain forms and is secreted intracellularly into subcellular fractionation observed (Fig. 8). Since only trace amounts of partially degraded MPOΔpro are detected in dense fractions, the results suggest that degradation of non-secreted MPOΔpro preferentially takes place in a pre-granule compartment. However, it is possible that substantial degradation of MPOΔpro would still take place in granule-like vacuoles but too rapidly to be detectable. Therefore, to rule out considerable transfer to granules with degradation, experiments were performed with chloroquine and NH₄Cl, agents that block lysosomal proteolysis. In particular, chloroquine blocks late proteolytic processing of MPO (16). Biosynthetic radiolabeling of MPOΔpro in transfected 32D cells and chase of the radiolabel in the presence of NH₄Cl or chloroquine does not diminish the degradation rate of MPOΔpro, indicating that lysosomes are not involved to any great extent in the degradation observed (Fig. 8). The secretion of MPOΔpro also occurs...
during incubation with NH$_4$Cl and chloroquine, although it is reduced by NH$_4$Cl. Secreted MPO$_{\Delta pro}$ is of slightly higher molecular weight than the protein retained intracellularly both in the control and in the presence of NH$_4$Cl or chloroquine, indicating additional glycosylation during the secretory process. To characterize the localization of the degradation further, cell radiolabeling experiments were performed in the presence of brefeldin A (BFA), which induces the disassembly of the Golgi complex, thus blocking ER-Golgi transport (35, 36). As expected, BFA blocks the secretion of MPO$_{\Delta pro}$ completely, but total degradation is observed with time (Fig. 8), indicating that degradation of MPO$_{\Delta pro}$ can occur in a pre-Golgi compartment. A higher molecular weight form of MPO$_{\Delta pro}$ is observed with time in the presence of BFA (Fig. 8). This form, which is also degraded, is probably the result of aberrant glycosylation in the presence of BFA.

Secreted MPO$_{\Delta pro}$ Is Resistant to Endo-H Indicating Complex Oligosaccharide Side Chains—Both intracellular pro and mature MPO are normally sensitive to digestion with Endo-H indicating the presence of high mannose oligosaccharide side chains (16, 17, 20). Consistent with published data, the intracellular forms of pro and mature MPO in 32D cells transfected with wild type MPO both show sensitivity to digestion with Endo-H indicating the presence of high mannose groups (Fig. 9A). However, an additional reduction in size is observed for the large subunit of mature MPO upon digestion with N-glycanase as compared with digestion with Endo-H. To ensure that complete digestion had taken place with Endo-H and Endo-N, the concentration of glycosidase were varied (Fig. 9A). The results show that the molecular mass is reduced by 4.5 kDa upon complete digestion with Endo-H and by 12.5 kDa upon complete digestion with N-glycanase (mean values from two separate experiments). Thus, the large subunit contains Endo-H-resistant oligosaccharides indicating the presence of complex oligosaccharides. The presence not only of high mannose but also of complex oligosaccharides in the large subunit of MPO has for technical reasons been overlooked in previous studies. We observed both high mannose and complex oligosaccharides also in the large MPO subunit of HL-60 cells that normally produce MPO (data not shown). Also the secreted pro-MPO shows partial Endo-H resistance both in the 32D cells transfected with MPO (Fig. 9A) and in HL-60 cells (data not shown). This indicates that the secreted pro-MPO achieves some complex mannose groups during passage through the Golgi compartment during constitutive secretion. The secreted pro-MPO shows heterogeneity, and removal of all oligosaccharides with N-glycanase reveals at least two distinct protein forms that differ in molecular mass (Fig. 9A). The smaller protein form is resistant and the larger is sensitive to Endo-H. Two distinct protein forms are also observed for secreted pro-MPO in the HL-60 cell line (data not shown).

The glycosylation pattern for MPO$_{\Delta pro}$ is shown in Fig. 9B. Intracellular MPO$_{\Delta pro}$ is highly sensitive to digestion with Endo-H. In contrast, the secreted MPO$_{\Delta pro}$ is homogeneous and Endo-H-resistant corresponding to the presence of complex oligosaccharide side chains. As judged by results from digestion with N-glycanase, the secreted form contains slightly more carbohydrate than the intracellular form consistent with processing into complex forms.

**DISCUSSION**

Neutrophils carry at least the following three types of granules: azurophil, specific, and gelatinase granules (37). Unique constituents of azurophil granules such as MPO and serine proteases are stored in enzymatically active forms, whereas proteases of specific and gelatinase granules are stored in
inactive forms to become activated first after exocytosis (1). Thus, azurophil granule enzymes are activated prior to storage, e.g., by removal of an activation peptide from proforms of the serine proteases (38). The activation peptide keeps enzyme activity latent and is removed as a late step during intracellular trafficking. Likewise, the propiece of prodefensin is removed before storage in azurophil granules of mature antibacterial defensin, which is non-catalytic. In this case the propiece is essential for subcellular trafficking and sorting, e.g., by interaction with a complementary hydrophobic part of mature defensin peptide or with a chaperone protein that facilitates transit and protects against adverse effects of the mature peptide (3). MPO, on the other hand, is enzymatically active prior to proteolytic removal of its propiece (1). Therefore, the propiece of pro-MPO probably does not play a role in protection against peroxidation during intracellular travelling unless it interacts with other molecules for this purpose. Rather, the propiece may play a role in conformational stability, retention, and/or sorting. The results of the present work are viewed in this context.

The murine myeloid 32D cell line was successfully employed for investigation of MPO synthesis. Thus 32D cells stably transfected with the cDNA for human MPO demonstrate the normal characteristics of MPO synthesis. Heme is incorporated into apoprotein-MPO resulting in production of pro-MPO that is processed into mature heterodimeric protein targeted to granule-like vacuoles of 32D cells. The same cell line has previously been utilized for the investigation of the posttranslational processing of human neutrophil defensin (3) and neutrophil serine proteases (5, 32). Previous attempts to use Chinese hamster ovary cells (39–41), baby hamster kidney cells (42), or baculovirus-infected Sf9 cells (43) for expression of MPO cDNA have not resulted in processing of the protein product. But, recently the human erythroleukemia K562 cell line transfected with MPO cDNA showed the typical processing seen during biosynthesis of MPO in myeloid cells (44).

What do our results reveal about the role of the propiece for post-translational processing, targeting, and secretion of MPO? Propeptide-deleted pro-MPO (MPOpro) was found to lack processing into mature light and heavy chain MPO and primarily became secreted to the exterior or degraded in a pregranule compartment. Therefore, the propiece is necessary for subcellular trafficking. The finding of continued degradation of MPOpro in the presence of lysosomotrophic agents and BFA rules out that the observed degradation should, after all, take place upon transfer to granules but too rapidly to be detectable. The results obtained for MPO processing can be compared with those for the lysosomal hydrolase cathepsin D, in which the precursor domains are indispensable for the formation of a stable proenzyme (45). Thus, in the latter case the propeptide appears to be necessary for the correct folding of the proenzyme that is required for trafficking. However, it was not possible to prove a direct role for the propeptide of cathepsin D in sorting, because the propeptide when attached to a secretory protein, α-lactalbumin, did not redirect it for lysosomes indicating that the propeptide might not be necessary for the sorting process as such (45). If a sorting machinery were to recognize precursors rather than mature peptides, propiece-deleted pro-MPO when available for sorting would be secreted instead of being sorted for storage in granules. This seems to be consistent with the finding that a large part of MPOpro is secreted, whereas almost none is transported to granules. However, a part of MPOpro is retained, most likely in the ER, and degraded. Proteasomes may have a role, although unproven, in proteolysis of MPOpro. One theoretical explanation is misfolding; if the propiece were required for folding, misfolding of a propiece-deleted pro-MPO might lead to retention in the ER.

For investigation of MPO processing into mature heterodimeric protein targeted to granule-like vacuoles of 32D cells, the same cell line has previously been utilized for the investigation of the posttranslational processing of human neutrophil defensin (3) and neutrophil serine proteases (5, 32). Previous attempts to use Chinese hamster ovary cells (39–41), baby hamster kidney cells (42), or baculovirus-infected Sf9 cells (43) for expression of MPO cDNA have not resulted in processing of the protein product. But, recently the human erythroleukemia K562 cell line transfected with MPO cDNA showed the typical processing seen during biosynthesis of MPO in myeloid cells (44).
fore, the propeptide of pro-MPO may promote resistance to mannosidases and/or glycosyltransferases whose action is required for production of complex mannose groups in a late Golgi compartment. However, results from baby hamster kidney cells transfected with MPO have shown that secreted pro-MPO contains at least one Endo-H-resistant oligosaccharide indicating the presence of complex mannose groups (42). Thus the presence of the propeptide does not prevent generation of complex oligosaccharides totally. Likewise, the present results show that secreted pro-MPO contains complex mannose groups which must have been added at a rapidly transient step as they are not detectable in cellular pro-MPO. That MPO\textsubscript{pro} lacks targeting to granules and is instead conveyed to the secretory pathway with concomitant synthesis of complex mannose groups during passage of trans-Golgi. The finding of some complex mannose groups in secreted pro-MPO also indicates that at least part of it has travelled the secretory pathway through trans-Golgi. The secreted pro-MPO consists of at least two protein forms with different molecular masses easily seen after removal of carbohydrate with N-glycanase and only the smaller one contains complex mannose groups. Simillar extracellular pro-MPO forms were observed in supernatants from HL-60 cells (data not shown) indicating that their occurrence may be a general phenomenon. It is possible that only the two forms have arrived at the cell surface through separate routes. The higher molecular mass component might have come through a secretory path excluding trans-Golgi and lacking complex mannose groups, whereas the lower molecular mass form might have come through another path. We speculate that the latter path is that for processing and storage of MPO but that it is linked to the secretory pathway at a distal point. Thus, the secreted lower molecular mass species could represent an intermediate MPO processing form that is in part released to the secretory pathway from an acidic pregranule compartment in which intermediate processing forms have been suggested to be produced (19, 22). Final processing occurs later (in granules) when escape to the secretory pathway is blocked. Intermediate MPO processing might take place in late acidic endosomes after receiving contents, including pro-MPO, from Golgi-derived vesicles. Because late endosomes are involved in transport in and out of the cell, it is possible that some material delivered to late endosomes escapes to the outside. An additional unproven possibility is that secreted pro-MPO, but not MPO\textsubscript{pro}, can re-enter the cell through receptor-mediated uptake into the endocytic pathway with transport to late endosomes and granules for processing and storage.

Acquisition of heme by heme-free apopro-MPO seems to be a rate-limiting step in subsequent processing into mature MPO of hematopoietic cells (11–13). The calcium-binding calreticulin, present in the ER of many cells, was shown to interact specifically with fully glycosylated apopro-MPO during a relatively short period early in MPO synthesis and not with heme-containing pro-MPO or mature MPO (14). These data suggest a role of calreticulin as a molecular chaperone facilitating heme insertion after which the calreticulin-MPO precursor complex dissociates and pro-MPO can leave the ER for further processing and targeting. Our results show that pro-MPO and MPO\textsubscript{pro} both have incorporated heme although the relative efficiency of incorporation is lower for MPO\textsubscript{pro}. In any case, the lack of propeptide may not block the interaction between apopro-MPO and calreticulin that is proposed to be necessary for heme incorporation (14). The propeptide seems not to play a major role for the initial processing of the translational product but rather plays a role later in processing and trafficking.

Finally, our results provide novel information on MPO biosynthesis, processing, and targeting. Elimination of the propeptide from pro-MPO blocks the maturation process, allows secretion, but abolishes accumulation of the final product for storage, suggesting a critical role of the propeptide for late processing of pro-MPO.

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