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Proconvertase proteolytic processing of an enzymatically active myeloperoxidase precursor

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Abstract

Optimal and efficient killing of ingested microbes by human neutrophils is mediated in large part by the action of hypochlorous acid produced by the myeloperoxidase-H₂O₂-chloride system in phagosomes. Myeloperoxidase gene transcription is limited to early myeloid precursors in the bone marrow, when myeloperoxidase is synthesized and stored in azurophilic granules for subsequent release from stimulated neutrophils. Promyeloperoxidase, the 90-kDa myeloperoxidase precursor synthesized in the endoplasmic reticulum (ER), contains a 125-amino acid pro-region whose function and fate during myeloperoxidase biosynthesis are unknown. Promyeloperoxidase has two fates during myeloperoxidase biosynthesis; the majority undergoes proteolytic processing to generate mature myeloperoxidase, while the remainder is constitutively secreted from the cells in bone marrow. We used a promyelocytic cell line that produces endogenous myeloperoxidase as well as human embryonic kidney cells stably expressing normal and mutant forms of myeloperoxidase to examine proteolytic processing of promyeloperoxidase. We demonstrated that CMK-RVKR, an inhibitor of subtilisin-like proteinases, blocked cleavage of the pro-peptide of promyeloperoxidase in a post-ER compartment. Mutants with alanine substitution of basic residues in the predicted proteinase cleavage site failed to undergo maturation to normal myeloperoxidase subunits and were arrested at the promyeloperoxidase stage. Whereas specific mutants varied as to their stability, secreted promyeloperoxidase from the mutants retained the capacity to generate hypochlorous acid. Taken together, these studies demonstrate proconvertase-dependent cleavage of promyeloperoxidase as an essential step in normal proteolytic processing and granule targeting of myeloperoxidase. Furthermore, although mutations in the proteinase cleavage site reduced intracellular stability of the mutants, the integrity of the heme group was not compromised, as chlorinating activity was retained in the secreted promyeloperoxidase.

Keywords

myeloperoxidase; proproteins; protein processing; biosynthesis

Introduction

Production and targeting of myeloperoxidase (MPO)¹ to azurophilic granules occur in promyelocytic myeloid precursors in normal human bone marrow [1–3]. Recapitulated in

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¹Abbreviations used: CHO, Chinese hamster ovary; CLN, calnexin; CMK-RVKR, Decanoyl-Arg-Val-Lys-Arg chloromethylketone; CRT, calreticulin; HEK, human embryonal kidney; MPO, myeloperoxidase; proMPO, promyeloperoxidase.

cultured human myeloid cell lines [3, 3–8] or heterologous transfections in K562, CHO, or HEK cells [9, 10], biosynthesis of mature MPO encompasses a series of critical steps [reviewed in [11]]. Under normal conditions, apoproMPO acquires heme in the endoplasmic reticulum (ER), thereby becoming enzymatically active 90-kDa proMPO [3, 12]. Heme acquisition in the ER is the rate-limiting step in MPO biosynthesis, as inhibiting heme synthesis blocks proteolytic processing of MPO precursors [12–14] and disruption of the Golgi network by culturing cells in the presence of brefeldin A arrests MPO maturation at the proMPO stage [12]. In a cellular compartment distal to the ER [12], the 90-kDa proMPO undergoes sequential proteolysis to a ~75 kDa intermediate species [8, 15–17] that is subsequently processed to the heavy and light subunits of mature MPO [Figure 1 and reviewed in [11]]. Although little is known about the function or fate of the pro-peptide of MPO, it does not serve as a sorting determinant that directs proMPO selectively to azurophilic granules [18, 19] and its deletion results in an arrest in processing [20]. Although most of the proMPO synthesized in the ER undergoes proteolytic processing and targeting to azurophilic granules, a substantial fraction is constitutively secreted by normal bone marrow granulocyte precursors [3] as well as by cultured human myeloid cell lines [3, 4, 16, 19–23] and heterologous lines expressing recombinant MPO [9, 10].

We present evidence that processing of proMPO to the 75-kDa intermediate species is mediated by a member of the proprotein convertase family of proteinases, similar to those that promote activation of peptide hormones and neurotransmitters [24, 25]. Mutations in the propeptide that compromised processing did not affect the functional integrity of the heme group, as the chlorinating activity of the secreted proMPO was preserved in the mutants.

Materials and Methods

Human embryonal kidney 293 (HEK) cells (CRL-1573) were obtained from American Type Culture Collection (Manassas, VA). The vector pcDNA3.1 and the antibiotic G-418 sulfate were obtained from Invitrogen (Carlsbad, CA). [³⁵S]-methionine/cysteine (Easy Tag Express ³⁵S Protein Labeling Mix; 37.0 TBq/mmol; 11 mCi/ml) was obtained from Perkin-Elmer (Waltham, MA). All tissue culture reagents were obtained from the Hybridoma Facility at the University of Iowa. Decanoyl-Arg-Val-Lys-Arg chloromethylketone (CMK-RVKR) was obtained from Bachem (Torrence, CA). Antibody against calnexin was obtained from Stressgen Bioreagents (Ann Arbor, MI). The monospecific rabbit antibody against human MPO was generated in our lab and previously described [26], as was the rabbit antibody against human calreticulin [27] that is also available commercially from Affinity Bioreagents (Golden, CO). Unless otherwise specified, all other reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Stably transfected cells lines

HEK cells were maintained in Dulbecco's modified Eagle's medium/Ham's nutrient mixture F12 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 100 mM HEPES, and 2 mM L-glutamine. As done previously [10, 28, 29], we used PCR to create specific mutations in normal MPO cDNA for stable heterologous expression in HEK cells, which are devoid of endogenous MPO. Sequencing of the cDNA prior to transfection was used to confirm the presence of the desired mutation and absence of unintended mutations. Once the sequence was confirmed, the mutant construct was cloned into pcDNA3.1 (-) and stable transfectants selected by G-418 sulfate and cloned by limiting dilution.

MPO biosynthesis by transfectants

Stably transfected HEK cells were used to examine biosynthesis of wild-type and mutant forms of MPO, as done previously [10, 27–30]. In summary, cells were grown at low density in medium supplemented with 2 µg/ml hemin for 24 hours prior to biosynthetic radiolabeling and then placed in methionine-free medium supplemented with hemin, dialyzed fetal bovine serum and antibiotics for 1 hour prior to pulse labeling with [³⁵S]-methionine/cysteine. After the indicated period of biosynthetic radiolabeling, cells were recovered or chased by the addition of cold methionine (1000-fold excess), before solubilization for subsequent analysis. Radiolabeled cells were solubilized and used in immunoprecipitations as previously described [10, 28, 29].

Biosynthetically radiolabeled MPO-related proteins were recovered from cell lysates or culture medium by immunoprecipitation with monospecific, polyclonal rabbit anti-human MPO, as previously described [10, 12, 19, 21, 22, 27–31]. Radiolabeled MPO-related proteins were separated by SDS-PAGE followed by autoradiography and quantitated by direct measurement of radioactivity using a phosphorimager (Typhoon 9410, GE Healthcare). MPO-related species that were associated with calreticulin (CRT) or calnexin (CLN) were recovered and quantitated using sequential immunoprecipitations, as previously described [27, 29, 30]. The CRT- and CLN-associated MPO species were separated by SDS-PAGE and quantitated using the phosphorimager.

Recovery of secreted proMPO

For analysis of secreted proMPO, spent culture media was collected from HEK cells, both wild-type and transfectants, after cells reached ~80% confluence in T162 culture flasks. Spent media (40 ml) was centrifuged (200×g, 6 min, 4°C) to remove cells, diluted 1:1 with 10 mM Tris-HCl (pH 7.4), and incubated with SP Sepharose Fast Flow beads (GE Healthcare) that had been washed in 10 mM Tris-HCl (pH 7.4). Typically, 50 µl of washed beads were added to 5 ml of spent media diluted with 5 ml of 10 mM Tris-HCl (pH 7.4). Beads and diluted medium were tumbled 4 hrs at 4°C and pelleted. Beads were resuspended in 1 ml Tris-buffered saline (TBS, pH 7.4) and washed 3 times in TBS (1 ml each wash). Washed beads were resuspended in 200 µl 1.5 M NaCl in Tris-HCl (pH 7.4) and tumbled at 4°C for 20 min. Previous experiments had demonstrated that 1.5 M NaCl/Tris-HCl eluted all of the bound proMPO from the beads. Beads were pelleted, and the supernatant transferred to an Amicon Ultracel (30K) and diluted with 3.5 ml phosphate buffered saline (PBS). The volume was reduced to 500 µl by centrifugation, and the dilution and concentration steps were repeated twice more. The final sample was adjusted with PBS to 500 µl and the protein concentration determined using Pierce BCA protein assay before being stored at 4°C until enzymatic activity was assayed.

The amount of MPO-related protein recovered as secreted proMPO was determined by immunoblotting. Samples were subjected to SDS-PAGE and blotting, as previously described, with blots incubated with MPO antibody (1:20,000 dilution) followed by donkey anti-rabbit-antibody conjugated with horseradish peroxidase (1:50,000) and processed with Pierce West femto chemiluminescence reagent. The amount of chemiluminescent signal was directly measured using a Typhoon 9410 phosphorimager.

MPO-dependent chlorinating activity

The chlorinating activities of the expressed wild type or mutant MPO were quantitated. Non-transfected cells and transfectants expressing normal or mutant MPO were cultured in the presence of 2 µg/ml hemin for 24–48 hours prior to recovery and solubilization for determination of enzymatic activity. MPO-specific chlorinating capacity was quantitated using a sensitive and specific assay for detection of HOCl-dependent generation of taurine

monochloramine [32], with a standard curve for HOCl production generated using purified MPO (0–500 fmoles).

HEK cells, both wild-type (negative control) and transfectants were treated with diisopropylfluorophosphate (1 mM) for 20 minutes and sonicated for 10 seconds on ice. Cell sonicates were centrifuged (228×g) to remove intact cells and the supernatant spun (10,800×g) to recover the membrane-bound compartment containing normal or mutant MPO, as previously described [10]. The pellet was subjected to three sequential freeze-thaw cycles in phosphate buffered saline (without calcium or magnesium) with 0.3% cetyltrimethylammonium bromide and 1% protease inhibitor cocktail. The solubilized pellet was clarified by centrifugation (50,000 × g, 10 min, 4°C, in TL 120.2 rotor) and the clarified contents used in assays. Data are expressed as nmoles of HOCl produced and all assays were performed in triplicate and each experiment was performed at least three times.

Results and Discussion

Sequential proteolytic events in processing of proMPO

The important role for MPO in optimal neutrophil antimicrobial activity and the unique chlorinating action of the MPO-H₂O₂-chloride system [reviewed in [33]] provide compelling incentives to define steps in the normal biosynthesis of this heme enzyme. In an effort to identify the proteinases that contribute to the processing events during MPO biosynthesis, HEK cells stably expressing normal MPO [HEK-MPO [10]] were biosynthetically radiolabeled in the absence or presence of three different cysteine protease inhibitors, N-acetyl-leucyl-leucyl-methionine, N-acetyl-leucyl-leucyl-norleucine, and E-64 (Figure 2). Whereas control cells produced mature MPO after 20 hrs of chase, cells cultured in the presence of inhibitors produced only the 75-kDa MPO intermediate, suggesting that cysteine protease inhibition terminated proper processing. As anticipated from previous work [12], the removal of the pro-peptide from proMPO occurred in a post-ER compartment, since no accumulation of the 75-kDa intermediate was detected in brefeldin A-treated cells cultured in the presence of E-64 (Figure 2). In contrast to the effects of the cysteine protease inhibitors, MPO biosynthesis by HEK-MPO cells cultured in the presence of CMK-RVKR, a specific inhibitor of proprotein convertases [34], generated only proMPO; processing of the 90-kDa proMPO to the 75-kDa biosynthetic intermediate of MPO was inhibited (Figure 2). Taken together, these findings demonstrate that two sequential steps occurred in the proteolytic maturation of normal MPO; proprotein convertases cleaved proMPO to the 75-kDa intermediate, and cysteine proteases catalyzed the processing of the 75-kDa species into mature MPO.

Potential targets in proMPO for proprotein convertases

Peptide and hormone precursors undergo proteolytic processing by enzymes belonging to the subtilisin-like proprotein convertase family [24]. These endoproteases cleave target proteins on the C-terminal side of basic residues presented in the motif [K/R]-[X]_n-[K/R]_↓, where X is any amino acid (although rarely cysteine), K/R indicates arginine or lysine, and n is 0, 2, 4 or 6 residues [24, 25, 35]. The amino acid sequence of proMPO includes the region ¹²⁸RKLRSLWRR¹³⁶ that encompasses the motif for a proprotein convertase substrate with several potential cleavage sites depending on different spacing between the basic residues (Figure 3A). In order to determine if these sequences of proMPO provide a target for proprotein convertases, we mutated all the basic residues in this region to alanines and generated an HEK cell line (R/K → A cells) stably expressing MPO containing ¹²⁸RALASLWAA¹³⁶ in place of the normal sequence. After pulse-chase radiolabeling, only the 90-kDa species was detected after 20 hrs of chase (Figure 3B), indicating that processing of proMPO to mature MPO in R/K → A cells was arrested. To

assess the interactions of the mutant MPO precursor with molecular chaperones known to participate in MPO biosynthesis [27, 30], sequential immunoprecipitations for ER chaperones calreticulin (CRT) and calnexin (CLN) were performed at 0 to 4 hours of chase. In contrast to normal apoproMPO and proMPO [27, 30], mutant MPO precursors failed to interact with CRT and exhibited a prolonged association with CLN (Figure 3C). Taken together, these observations support the hypothesis that ¹²⁸RKLRS¹³⁶WRR included sites for endoprotease removal of the pro-peptide from proMPO, as the 90-kDa MPO precursor did not undergo proteolytic cleavage to generate the 75-kDa intermediate in R/K → A transfectants. Because the failure of R/K → A to interact normally with ER molecular chaperones raised concerns that the mutations may have resulted in structural or conformational changes more extensive than simply disrupting the target for cleavage, we generated stable transfectants with mutations of individual basic residues in the region of interest.

Individual mutations in putative target for proMPO proprotein convertases

Stable HEK transfectants expressing alanine substitutions for basic amino acids at residues 129, 131, 135, and 136 in normal MPO were created and analyzed for their capacity to undergo normal proteolytic processing to mature MPO and to be enzymatically active. Biosynthetically radiolabeled K129A cells, a cell line with lysine at codon 129 replaced by alanine, synthesized a 90-kDa MPO-related protein that did not undergo proteolytic maturation to the 75-kDa intermediate or the subunits of mature MPO (Figure 4). The K129A mutation did not compromise exit of the protein from the ER, as the secreted pro-form of K129A was recovered from the culture medium, (Figure 3B), just as normal MPO and the R/K → A mutant were.

Similar results were obtained with clones expressing mutations in the other three residues of interest. Transfectants expressing R131A, R135A, or R136A each synthesized a 90-kDa MPO species that was secreted but failed to undergo normal proteolytic processing to the 75-kDa intermediate or subunits of mature MPO (Figure 4). We speculated that processing of MPO precursors and failure to generate mature MPO could reflect a failure to incorporate heme properly [12–14], ER-associated degradation of misfolded intermediates [29], failed proteolytic cleavage, or some combination of each of these events.

Consequences of mutations on generation of mature MPO

Mature MPO is composed of two identical protomers, with each protomer containing a 59-kDa heavy subunit and a 13.5-kDa light subunit, bound together by a single disulfide bond [36–38]. Consequently, we reasoned that determination of the distribution of MPO-related species between 90-kDa and 59-kDa forms during pulse-chase biosynthetic radiolabelling provides an index of successful proteolytic maturation of MPO precursors. Whereas the ratio of 90-kDa to 59-kDa for normal MPO was $0.48 \pm .05$ ($n = 18$), the ratio was higher in the mutant cell lines (Table 1), suggesting that each of the mutations compromised to varied degrees productive conversion of precursor to mature MPO. However, we recognize that the 90:59 ratio alone may not reflect all the consequences of the mutations, as alterations in the pro-peptide of MPO could influence the fates of apoproMPO and proMPO in several different ways (Figure 1). Improper folding of apoproMPO could compromise heme acquisition and result in ER retention and proteasomal degradation, as occurs with the Y173C mutation in one form of inherited MPO deficiency [29]. Misfolding of proMPO could undermine normal proteolytic maturation [28], constitutive secretion of proMPO, or both.

To understand better the impact of these specific mutations in the pro-region on the fate of newly synthesized MPO species, we quantitated the 90-kDa MPO protein in cells

immediately after biosynthetic radiolabeling (*i.e.* 0 hr chase) and in cells after 20 hrs of chase, reasoning that differences in cell-associated MPO precursor at zero and 20 hours would reflect in part the relative fates of newly synthesized mutant MPO precursors. The relative amount of cell-associated 90-kDa MPO precursor, expressed as the percent of that at zero hours chase, was 31.9 ± 3.7 ($n = 11$) for the cell line expressing normal MPO. Although variable for the mutants (Table 1), all but R131A were greater than the wild-type value. In contrast, both the residual 90-kDa and the 90:59 were highest for K129A. Inhibition of proteasome activity by MG132 (17 μ M) increased the amount of cell-associated 90-kDa species in K129A cells, but the rescued 90-kDa was not processed normally, as there was no concomitant increase in the 59-kDa subunit after 20 hours of chase (data not shown), suggesting that the rescued 90-kDa precursor was not processed normally. Taken together, these data demonstrate that the mutations in the pro-peptide of MPO blocked normal maturation in all cases but had varied effects on the relative stability of cell-associated proMPO.

Consequences of proMPO mutations on activity of cell-associated and secreted MPO species

Normal proMPO has two potential fates as it exits the ER: most undergoes sequential proteolytic processing to the mature form of the protein packaged in azurophilic granules, while the rest remains intact and is secreted constitutively. In normal bone marrow myeloid precursors [3], cultured human myeloid cell lines [3, 4, 16, 19, 20, 22, 23], and our HEK expression system [10], some of the newly synthesized proMPO retains the pro-peptide and is constitutively secreted.

To determine the extent to which these mutations undermined heme-dependent enzymatic activity, we measured the capacity of both cell lysates and secreted proMPO isolated from conditioned culture media to chlorinate, an activity unique to MPO. The cell-associated chlorinating activity of all mutants was significantly less than that of transfectants expressing normal MPO. However, it is difficult to clearly assess the impact of the specific mutations on chlorinating activity since the MPO-related species in lines expressing normal and mutant protein are different. In the case of transfectants expressing normal MPO, *both* proMPO and mature MPO contribute to cell-associated enzymatic activity, whereas only the former exists in the mutants cells, since proteolytic processing was arrested (Figure). In order to obtain a less ambiguous view of the effects of the specific mutations on the function of the heme group, we measured the chlorinating capacity of secreted proMPO recovered from culture medium. Both normal and mutant secreted proMPO generated HOCl, with normal proMPO producing 10.41 ± 0.36 nmoles HOCl per μ g protein (Table 2). With the exception of K129A, the mutants exhibited normal chlorination, indicating that most of mutations in the region did not undermine heme activity.

Conclusions

Taken together, these studies demonstrate several previously unknown features of MPO biosynthesis. First, the conversion of proMPO to mature MPO involves two sequential steps. A subtilisin-like proprotein convertase cleaves the pro-peptide, thereby generating a 75-kDa intermediate species. Mutation of the proprotein cleavage sites blocked processing and resulted in unstable forms intracellularly. Second, production of the 75-kDa intermediate species previously reported to give rise to mature MPO subunits [8, 15–17] was blocked by cysteine protease inhibitors, although the specific enzyme responsible for processing was not identified. Third, secreted proMPO exhibited chlorinating activity, in line with detailed analyses of the recombinant monomeric MPO-related protein expressed in CHO cells [39–41]. Mutations that inhibited proteolytic processing had no significant effect on the capacity of secreted proMPO forms to support chlorination, with the exception of the K129A mutant.

An earlier report noted the presence in normal human plasma of an 85-kDa protein with immunoreactivity with MPO antiserum [42], and the authors speculated that it might represent an MPO precursor, although neither the identity nor the enzymatic activity of that protein was assessed. Although we did not directly examine the relationship between the immunoreactive protein found in normal plasma and the proMPO secreted in our system, our work and the studies of others are consistent with the circulating protein being proMPO arising from myeloid precursors in normal bone marrow. The presence of functional proMPO circulating in plasma would have important and clinically relevant implications. Mature MPO catalyzes the generation of reactive oxidant species that mediate a wide variety of structural changes, including post-translational modifications of proteins, peroxidation of lipids, depletion of NO[•], and formation of NO[•]₂ [43, 44]. Although there are several inflammatory disorders where such biochemical reactions might occur, extensive epidemiologic and experimental data link MPO-mediated biochemistry with the full range of cardiovascular disease [45], from acute coronary events [46] to dysrhythmias [47] to the pathogenesis of atherosclerosis [reviewed in [48]]. Precise definition of the full range of activities of proMPO, susceptible substrates in plasma and of the consequences for the initiation and promotion of inflammatory cardiovascular disease remain to be determined.

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FIGURE 1. Schematic of proteolytic events in processing of MPO

The 80-kDa primary translation product for MPO undergoes cotranslational cleavage of the pre sequence and glycosylation in the ER to generate the 90-kDa glycosylated precursor that interacts transiently with resident chaperones calreticulin (CRT) and calnexin (CLN). The heme-free apoproMPO acquires heme in the ER, thereby becoming proMPO, which has two subsequent fates after exiting the ER. Most undergoes stepwise proteolytic processing to form a ~75-kDa intermediate that subsequently has endoproteolytic removal of a hexamer (ASFVTG) to produce the subunits of mature MPO. The remaining proMPO enters the secretory pathway (dotted line) and is constitutively released from the cell.

FIGURE 2. Effects of protease inhibitors on proteolytic processing of proMPO

MPO-species were immunoprecipitated from cultured PLB-985 cells biosynthetically radiolabelled for one hour with a 20 hour chase in the absence (Control) or presence of protease inhibitors. *A*, The cysteine protease inhibitors N-acetyl-leucyl-leucyl-methionine (ALLM) and N-acetyl-leucyl-leucyl-norleucine (ALLnL) blocked the second step in proteolytic processing, resulting in accumulation of the 75-kDa intermediate species and inhibition of production of mature MPO (indicated by 59-kDa heavy subunit). *B*, In the presence of brefeldin A (BFA), proMPO failed to undergo any processing. Inhibition of the conversion of the 75-kDa to mature MPO by E-64, another cysteine protease, did not occur in the presence of BFA, indicating that proteolytic processing of proMPO occurred in a post-ER compartment. *C*, The proprotein convertase inhibitor CMK-RVKR blocked processing at the proMPO stage.

FIGURE 3. Potential proprotein convertase target in proMPO

A, The pro-peptide of MPO includes a sequence, $^{128}\text{RKLRSLWRR}^{136}$, that is consistent with the $(\text{K/R})-(\text{X})_n-(\text{K/R})\downarrow$ motif recognized by proprotein convertases. **B**, The R/K → A mutant of MPO, wherein all potential basic amino acid cleavage sites for proprotein convertases were replaced with alanine, synthesized proMPO that failed to undergo proteolytic processing. **C**, After one hour labeling and chase for 0 to 4 hours, the R/K → A mutant failed to associate with CRT and exhibited prolonged association with CLN. Data shown are representative of three independent pulse-chase experiments and individual sequential immunoprecipitations.

FIGURE 4. Effects of selected mutations in the pro-peptide of MPO on processing of proMPO
 Stable HEK transfectants expressing normal (MPO) or mutant MPO (K129A, R131A, R135A, and R136A) were pulse-labelled for one hour and chased for 20 hours before immunoprecipitation of cells or supernatant (medium) with MPO antiserum. The processing of proMPO to mature MPO was less than normal in each of the mutants, with the most extensive inhibition in K129A (see Table 1). Data shown are representative of 3–11 independent pulse-chase experiments and individual immunoprecipitations. Note that the same control panel is shown for R135A and R136A, as these three were analyzed together in the representative experiment selected to present.

Table 1
Processing of proMPO and mutant proMPO

Stably transfected HEK expressing normal or mutant MPO were biosynthetically radiolabelled and MPO-related proteins immunoprecipitated immediately after labeling (0 hr) and 20 hrs after chase. Immunoprecipitates were separated by SDS-PAGE and the amount of immunoprecipitated 90-kDa and 59-kDa MPO-related protein quantitated using a phosphorimager. The ratio of precursor to mature MPO (90-kDa: 59-kDa) and the percent of 90-kDa made in one hour and still present after 20 hrs chase are shown as mean $\pm$ SEM (number of experiments).

Source	90-kDa: 59-kDa (n)	Δ 90-kDa (0–20 hrs) % (n)
Normal MPO	0.48 $\pm$ 0.05 (18)	31.9 $\pm$ 3.7 (11)
K129A	2.47 $\pm$ 0.31 (8)	59.4 $\pm$ 7.6 (7)
R131A	0.90 $\pm$ 0.11 (6)	21.7 $\pm$ 3.2 (4)
R135A	1.77 $\pm$ 1.41 (3)	50.2 $\pm$ 6.6 (3)
R136A	1.77 $\pm$ 0.52 (3)	39.1 $\pm$ 0.4 (3)

Table 2
Chlorinating activity of normal and mutant cell-associated MPO and secreted proMPO species

Cell lysates and conditioned culture media from stably transfected HEK cells expressing normal or mutant MPO were assayed for the ability to generate HOCl. Data are expressed as nmoles HOCl produced/0.5 μ g of protein/30 minutes. Results are mean $\pm$ SD, and n = 6 for studies of cell-associated activity and n = 3 for studies of secreted proMPO. The chlorinating activity of only the K129A form of proMPO was significantly different from that of normal proMPO (p = .047).

	Cell-associated	Secreted proMPO
Normal MPO	11.00 $\pm$ 0.34	10.41 $\pm$ 0.36
K129A	0.13 $\pm$ 0.08	6.12 $\pm$ 0.60*
R131A	1.55 $\pm$ 0.05	10.04 $\pm$ 0.29
R135A	1.17 $\pm$ 0.17	9.56 $\pm$ 0.19
R136A	0.18 $\pm$ 0.08	9.57 $\pm$ 0.42