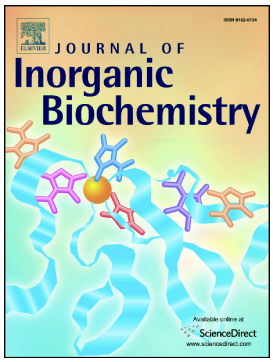


Catalase prevents myeloperoxidase self-destruction in response to oxidative stress

Iyad Ali, Sana N. Khan, Charalampos Chatzicharalampous, David Bai, Husam M. Abu-Soud

PII: S0162-0134(18)30707-4
DOI: https://doi.org/10.1016/j.jinorgbio.2019.110706
Article Number: 110706
Reference: JIB 110706

To appear in: *Journal of Inorganic Biochemistry*

Received date: 3 December 2018
Revised date: 17 April 2019
Accepted date: 2 May 2019

Please cite this article as: I. Ali, S.N. Khan, C. Chatzicharalampous, et al., Catalase prevents myeloperoxidase self-destruction in response to oxidative stress, Journal of Inorganic Biochemistry, https://doi.org/10.1016/j.jinorgbio.2019.110706

Catalase prevents myeloperoxidase self-destruction in response to oxidative stress

Iyad Ali[1,2], Sana N. Khan[1], Charalampos Chatzicharalampous[1], David Bai[1], Husam M Abu-Soud[1,3]*.

[1]*Department of Obstetrics and Gynecology, The C.S. Mott Center for Human Growth and Development, Detroit, MI 48201, USA*

[2]*Department of Biochemistry and Genetics, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus 7, Palestine*

[3]*Department of Microbiology, Immunology and Biochemistry, Wayne State University School of Medicine, Detroit, MI, 48201, United States.*

*Corresponding Author: Husam M. Abu-Soud, Ph.D, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, The C.S. Mott Center for Human Growth and Development, 275 E. Hancock Detroit, MI 48201, Tel. 313 577-6178, Fax. 313 577-8554, EMail: habusoud@med.wayne.edu

Running title: Hypochlorous acid and heme destruction

Keywords: MPO, Catalase, Respiratory inflammation, Heme destruction, Free iron, Antioxidants.

Abstract

Catalase (CAT) and myeloperoxiase (MPO) are heme-containing enzymes that have attracted attention for their role in the etiology of numerous respiratory disorders such as cystic fibrosis, bronchial asthma, and acute hypoxemic respiratory failure. However, information regarding the interrelationship and competition between the two enzymes, free iron accumulation, and decreased levels of non-enzymatic antioxidants at sites of inflammation is still lacking. Myeloperoxidase catalyzes the generation of hypochlorous acid (HOCl) from the reaction of hydrogen peroxide (H_2O_2) and chloride (Cl^-). Self-generated HOCl has recently been proposed to auto-inhibit MPO through a mechanism that involves MPO heme destruction. Here, we investigate the interplay of MPO, HOCl, and CAT during catalysis, and explore the crucial role of MPO inhibitors and HOCl scavengers in protecting the catalytic site from protein modification of both enzymes against oxidative damage mediated by HOCl. We showed that CAT not only competes with MPO for H_2O_2 but also scavenges HOCl. The protective role provided by CAT versus the damaging effect provided by HOCl depends in part on the ratio between MPO/CAT and the affinity of the enzymes towards H_2O_2 versus HOCl. The severity of such damaging effects mainly depends on the ratio of HOCl to enzyme heme content. In addition to its effect in mediating protein modification and aggregation, HOCl oxidatively destroys the catalytic sites of the enzymes, which contain porphyrin rings and iron. Thus, modulation of MPO/CAT activities may be a fundamental feature of catalysis, and functions to down-regulate HOCl synthesis and prevent hemoprotein heme destruction and/or protein modification.

Introduction

Catalase (CAT) and myeloperoxidase (MPO) are hemoproteins that have recently gained increasing attention for their potential role in the etiology of respiratory conditions related to oxidative stress [1-3]. Interestingly, many inflammatory disorders such as cystic fibrosis (CF), bronchial asthma, and acute hypoxemic respiratory failure, in which MPO/CAT are known to be elevated, and are also associated with significant free iron accumulation [4-9]. It has been postulated that variations in the levels or function of these two enzymes could modulate respiratory illness risk, especially under conditions of oxidative stress [1, 2]. However, a detailed understanding of the interrelationship and competition between the two enzymes at sites of inflammation is still lacking. Catalase is an anti-inflammatory enzyme that predominantly competes with MPO by converting hydrogen peroxide (H_2O_2) to H_2O and oxygen [10]. Myeloperoxidase, the most abundant protein in neutrophils and monocytes, catalyzes the reaction between chloride (Cl^-) and H_2O_2 to form hypochlorous acid (HOCl) [11-13]. Hypochlorous acid is a powerful oxidant, and is extremely reactive with the heme moiety of MPO and other hemoprotein model compounds causing heme damage and free iron release [14-17]. Hypochlorous acid when released from the MPO cavity, first diffuses in the solution as a whole, binds to MPO heme iron forming MPO-Fe(III)-OCl complex, and then medicates MPO heme destruction, precluding the enzyme from functioning at the maximum activity (80-90% inhibition) [14].

Accordingly, inhibition of MPO-chlorinating activity and/or factors that influence rates of HOCl removal following its synthesis by MPO are of significant interest. In the biological system, a major pathway for HOCl removal is through its interactions with antioxidant compounds such as thiols (glutathione (GSH), taurine, and methionine), carotenoid (lycopene), derivatives of

tetrapyrrole macrocyclic rings (heme and vitamin B_{12}), and indole compounds (melatonin and tryptophan) [18-24]. These molecules compete with Cl^-, the natural substrate of MPO, by serving as a one electron substrate of MPO Compounds I (ferryl porphyrin π cation radical, Fe(IV)=O(+π•)) and Compound II (ferryl complex; Fe(IV)=O), and/or directly scavenging HOCl [18-24].

Several respiratory diseases with underlying inflammation have been linked to abnormal MPO to CAT ratios, enhanced iron levels, and decreased levels of antioxidants [1, 2, 9, 25-27]. Increased MPO activity combined with decreased CAT activity creates an environment of increased reactive oxygen species (ROS) that are implicated in various cancers and in chronic respiratory inflammation [28]. Synthetic compounds with superoxide dismutase (SOD) and CAT activities have shown promising results in animal models against a variety of oxidant exposures including cigarette smoke in the lung [27].

In this work, we utilized H_2O_2-selective electrode analysis and direct spectroscopic measurements to determine the interdependence between CAT and MPO, and the role of HOCl in down-regulating the catalytic activity of both enzymes. Our recently published [14, 16, 17] and the current work, highlights the link between free iron accumulation, decreased levels of antioxidant, and the catalytic activity of both enzymes.

Materials and methods

Materials

All the materials used were of the highest-grade purity and used without further purification. Catalase powder from bovine liver, sodium hypochlorite (NaOCl), melatonin, L-methionine, and taurine were obtained from Sigma‑Aldrich (St. Louis, MO, USA).

MPO purification

Myeloperoxidase was initially purified from detergent extracts of human leukocytes by sequential lectin affinity and gel-filtration chromatography, as previously reported [29-31]. Trace levels of contaminating eosinophil peroxidase were then removed by passage over a sulfopropyl Sephadex column [30]. Purity of isolated MPO was established by demonstrating a Reinheitszahl (RZ) value of >0.87 (A_{430}/A_{280}), SDS‑PAGE analysis with Coomassie blue staining, and gel tetramethylbenzidine peroxidase staining to confirm no contaminating eosinophil peroxidase activity. Enzyme concentration was determined spectrophotometrically utilizing extinction coefficients of 89,000 M^{-1} cm^{-1}/heme of MPO at 430 nm [32]. Catalase, from the manufactures, was used without further purification. Catalase concentration was determined spectrophotometrically using extinction coefficients of 120,000 M^{-1} cm^{-1}/heme at 406 nm [33].

H_2O_2-selective electrode measurements

Hydrogen peroxide consumption was measured using an H_2O_2-selective electrode (Apollo 4000 free radical analyzer; World Precision Instruments, Sarasota, FL, USA). Experiments were performed at 25^{o}C by immersing the electrode in 3 ml of 0.2 M sodium phosphate buffer, pH 7.4. Hydrogen peroxide (10 μM) was added to a continuously stirred buffer solution containing fixed amount of MPO (40 nM, final) and various concentrations of CAT (40-160 nM), or fixed amount of CAT (40 nM final) and various concentrations of MPO (40‑160 nM, final), and/or Cl^- (100 mM) during which the change of H_2O_2 concentration was continuously monitored.

Absorbance measurements

The absorbance spectra were recorded using a Cary 100 Bio UV–visible photometer, at 25°C, pH 7.4. Experiments were performed in 1 ml phosphate buffer solution supplemented with fixed amounts of MPO (~0.6 μM) and Cl^- (100 mM) and increasing concentrations of H_2O_2 (0–200

μM), in the absence and presence of different ratios of CAT relative to MPO and/or various antioxidants (100 μM methionine, taurine, or melatonin).

HOCl preparation

Hypochlorous acid was prepared as previously described, with some modifications [34]. Briefly, a stock solution of HOCl was prepared by adding 1 ml of NaOCl solution to 40 ml of 154 mM NaCl and the pH was adjusted to around 3 by adding hydrochloric acid. The concentration of active total chlorine species in solution, expressed as $[HOCl]_T$ (where $[HOCl]_T=[HOCl]+[Cl_2]+[Cl_3^-]+[OCl^-]$) in 154 mM NaCl, was determined by converting all the active chlorine species to OCl^- by adding a bolus of 40 μl of 5 M NaOH and measuring the concentration of OCl^-. The concentration of OCl^- was determined spectrophotometrically at 292 nm (ε=362 M^{-1} cm^{-1}) [35] As HOCl is unstable, the stock solution was freshly prepared on a daily basis, stored on ice, and used within 1 h of preparation.

Results

Influence of HOCl generation on MPO/CAT catalytic functions

We first investigated if multiple rounds of H_2O_2 addition to the MPO/Cl^- system could affect MPO performance and if the generated HOCl would affect the MPO peroxidase activity. Fig 1, depicts an experiment in which six cycles of MPO activity were promoted by repeatedly adding a limiting amount of H_2O_2 (10 μM) to trigger transient HOCl synthesis in a single sample of MPO. The amount of H_2O_2 accumulated in the solution mixture (amplitude H_2O_2 signal) and the time of its consumption (duration of the signal) were continually measured after the addition of the H_2O_2 solution to a continuously stirred buffer containing MPO (40 nM)/Cl^- (100 mM). The first H_2O_2 addition to the enzyme solution led to an instant disappearance in the H_2O_2 signal due

to its consumption as a substrate during catalysis, indicating that MPO is catalytically active, similar to prior reports [14]. Sequential additions of the same amount of H_2O_2 to the reaction mixture inhibited MPO activity, as judged by the accumulation of H_2O_2 (indicated by the amplitude of the signal) from the decreased rate of H_2O_2 consumption (indicated by the duration of the signal) (Fig. 1A). This reduction in MPO activity required the buildup of sufficient amounts of HOCl generated by multiple rounds of H_2O_2 consumption. We also utilized the H_2O_2-selective electrode to show that CAT alone can rapidly consume H_2O_2. As shown in Fig. 1B, the first H_2O_2 (10 μM) addition to the enzyme solution led to an instant disappearance of H_2O_2 signal due to its consumption as a substrate by the enzyme during steady-state catalysis, indicating that CAT is catalytically active. However, sequential addition of the same amount of H_2O_2 to the reaction mixture demonstrated a similar instant consumption of H_2O_2 (Fig. 1B).

To determine whether CAT possesses the ability to compete with MPO on H_2O_2 and prevent HOCl-mediated heme destruction of both enzymes, identical experiments were repeated in the presence of both MPO and CAT. Experiments were carried out by either employing a fixed amount of MPO (40 nM) and varying concentrations of CAT (40, 80, 120, 160 nM) (Fig. 2A) or a fixed amount of CAT (40 nM) and varying concentrations of MPO (40, 80, 120, 160 nM) (Fig. 2B). In each MPO/CAT cycle, H_2O_2, the common substrate of both enzymes, was the limiting factor and would be reduced completely after each addition to the solution mixture by both enzymes. Remarkably, addition of H_2O_2 to a continuously stirred reaction mixture supplemented with 40 nM MPO, 100 mM Cl^-, and 40 nM CAT, demonstrated a modest brief increase in H_2O_2 signal, indicating rapid H_2O_2 disappearance (Fig. 1C). However, after several additions of H_2O_2 to the solution mixture the H_2O_2 signal rose rapidly, achieved its maximum level after ~30 s, and then gradually returned to baseline when all the H_2O_2 was consumed (Fig. 1C). Thus, the

accumulation of free H_2O_2 in MPO/CAT milieu requires more addition of H_2O_2 compared to the MPO alone (Fig. 1B). Area-under-the-curves (AUC), which reflect the maximum signal intensity and the duration of H_2O_2 consumption by both enzymes, were determined and plotted as a function of H_2O_2 concentration. In both cases, no significant increase in the AUC was observed when incremental addition of H_2O_2 was added to CAT alone (Fig. 2 A&B). MPO was not immediately inhibited by self-generated HOCl and remained active until a sufficient amount of HOCl was built-up in the solution mixture (Fig. 2 A&B). When a 1:1 ratio of MPO:CAT was used, this condition caused a right shift in the curve compared to MPO alone. Increasing CAT concentrations caused a further shift in the curves to the right (Fig. 2A). A similar trend was also observed by increasing the MPO to CAT ratio, but to a lesser degree (Fig 2B). Another factor that caused the MPO inactivation curve to shift to the right in addition to MPO:CAT ratio, is the presence of an HOCl scavenger.

Effect of MPO inhibitor and /or HOCl Scavengers

Melatonin, methionine, and taurine react with HOCl at rates from 3.4×10^7 to about 10^4 M^{-1} s^{-1} and have been used to scavenge HOCl or inhibit MPO chlorinating activity and protect MPO from inhibition and destruction by HOCl [36-38]. We therefore examined if these would protect MPO/CAT from HOCl in our system. Fig. 3B shows the effect of melatonin (100 µM) on MPO and CAT activities during HOCl synthesis. In this experiment, 10 µM (3µl) of H_2O_2 was added six consecutive times (at the arrow indicators in Fig. 3) to initiate six rounds of MPO/CAT catalysis at 25°C. Hydrogen peroxide accumulation and its decay were monitored as an increase and decrease in H_2O_2 signal over time. The amount of H_2O_2 accumulated in the solution mixture (amplitude of the H_2O_2 signals) and the time of its exhaustion reaction (duration of H_2O_2 signal)

were nearly identical within experimental error for all six trials. The data showed that consumption of H_2O_2 can occur at multiple times without slowing the rate of H_2O_2 consumption by MPO and CAT. Panel 3A is the control reaction, in which both MPO/Cl^- (40 nM/100 mM) and CAT (40 nM) were added to the reaction at time zero to initiate concurrent H_2O_2 consumption as H_2O_2 is added to the reaction (at the arrow indicators in Fig. 3) and subsequent HOCl synthesis. In this experiment (Fig. 3B), melatonin is a $1e^-$ substrate of MPO that competes with Cl^-, and this shifts the MPO catalysis paradigm from a $2e^-$ to a $1e^-$ oxidation pathway. As such, MPO consumed H_2O_2 more slowly without producing HOCl, and the H_2O_2 signal in the presence of melatonin correspondingly had much higher amplitude and lower decay rate relative to the control. Similar results have been observed for methionine and taurine (data not shown). Thus, these compounds, such as melatonin, methionine, and taurine, could also prevent HOCl-mediated heme destruction of both enzymes.

Effect of HOCl generation on MPO and CAT heme during steady-state catalysis

Our next experiments utilized light absorbance spectroscopy to examine if a significant amount of the MPO and CAT heme contents would be destroyed during catalysis of HOCl synthesis from MPO-Cl^--H_2O_2 system. Fig. 4 contains UV-Visible spectra of MPO alone (~0.6 µM) (dashed line), CAT alone (~0.6 µM) (dotted line), and MPO and CAT in combination (solid line). The starting MPO spectrum demonstrated a characteristic Soret peak at 430 nm with absorbance shoulders at 500 and 631 nm, indicative of ferric heme, whereas the absorption spectrum of the purified CAT exhibited a Soret peak at 406 nm and an additional minor peak at 610 nm. We next utilized the absorbance spectra to monitor MPO/CAT heme destruction during HOCl synthesis to see how HOCl levels correlate with MPO/CAT heme destruction. Enzyme

reactions reported here were run at 25°C in 1ml cuvettes, under conditions in which H_2O_2 was the limiting substrate and would be consumed completely by MPO/CAT after several minutes of reaction. For clarity, a cuvette containing phosphate buffer supplemented with CAT (~0.6 µM) and 100 mM Cl^- was mixed well and used to make the baseline of the absorbance spectra, from 300-500 nm, zero (Fig. 5 dotted line). The dashed line is the prominent Soret absorbance centered at 430 nm after addition of MPO (~0.6 µM) to the solution mixture (Fig. 5). Sequential incremental addition of 20 µM H_2O_2 (3 µL) to the solution mixture caused MPO and CAT heme destruction in a dose dependent manner, as judged by the loss of visible absorbance at 430 nm (MPO-Soret peak) and 406 nm (CAT-Soret peak) (Figs. 5A, 5B). Spectra were recorded immediately after the addition of H_2O_2 (Fig. 5A) as well as 10 min. after the addition of H_2O_2 (Fig. 5B) to demonstrate the slight accumulation of MPO Compound II as the stable form of MPO. To examine the potential physiological significance of these observations, we also determined the effect of HOCl scavengers on HOCl-mediated heme destruction utilizing similar methods. Fig. 5C shows the same experiment in the presence of methionine (100 µM), an MPO inhibitor and HOCl scavenger, which showed no alteration of MPO or CAT spectra, indicating that methionine completely prevented MPO/CAT heme destruction and/or modification. When similar experiments were performed with a MPO:CAT ratio of 5:1, less H_2O_2 was needed to achieve an increased degree of heme alteration, and at the conclusion of the experiment the MPO heme was completely destroyed, as indicated by flattening of the MPO Soret absorption peak at 430 nm (data not shown). Additionally, the buildup of the absorbance shoulder at 450 nm is identical to that partial formation of authentic MPO Compound II, a long lived intermediate, where its accumulation is the first step in HOCl-mediated heme destruction in hemoprotein [14, 16, 17]. Furthermore, when MPO:CAT ratio was 5:1, HOCl-mediated heme destruction was, to a

large extent, prevented in the presence of excess amounts of methionine (100 μM) with slight formation of MPO compound II. Methionine, like taurine and melatonin, is an important scavenger for HOCl that eliminates the impact of it on both CAT and MPO [36-38]. Similar experiments were also done using exogenously added HOCl to the enzyme solution mixtures; it showed the same effect, confirming that the alteration in the spectra of Fig. 5 comes from HOCl. Together, these results suggest that a significant amount of MPO and CAT were destroyed during aerobic HOCl synthesis under these conditions, and increasing the ratio between MPO and CAT increased the degree of heme destruction and/or modification, a process that was substantially inhibited by the presence of HOCl scavengers.

Finally, we utilized H_2O_2- selective electrodes to determine whether HOCl mediated MPO heme destruction occurs first and CAT destruction/modification second or vice versa. During continuous monitoring of H_2O_2 levels with an H_2O_2-selective electrode, rates of H_2O_2 consumption from the reaction were dramatically attenuated by addition of HOCl (10 μM) to MPO/CAT mixture with prior H_2O_2 addition to the solution mixture. Under these experimental conditions, the H_2O_2 signal rose rapidly, achieved a maximum after ~30 s, and fell gradually to the base line as H_2O_2 was depleted. To ensure MPO/CAT inactivation, an additional consecutive two HOCl aliquots (10 μM)/H_2O_2 (10 μM) were added to a continuously stirred phosphate buffer (200 mM, pH 7.4) supplemented with 40 nM MPO and 40 nM CAT at 25ºC. Here, the H_2O_2 signal rose rapidly, achieved a maximum after ~30 s, and fell very slowly not reaching the base line as H_2O_2 was depleted slowly. The reaction mixture then received 100 mM Cl^- followed by two consecutive H_2O_2 additions (denoted by the arrows). The fact that the addition of Cl^- led to acceleration of H_2O_2 consumption (Fig. 6) indicates that MPO is still active and that the slow reaction could be due to the formation of Compound II, the stable form of MPO, partial MPO

heme destruction, or a combination of both. Therefore, our experiment shows that CAT is inactivated prior to MPO in the presence of HOCl. Collectively, these results may suggest that CAT is more susceptible to HOCl-mediated destruction than MPO under these experimental conditions.

Discussion

Our central finding is that CAT competes with MPO for the substrate H_2O_2 and thus prevents HOCl-mediated heme degradation and/or protein modification. The protective role provided by CAT versus the damaging effect of HOCl depends on both the MPO/CAT ratio and the enzymes' affinity towards H_2O_2 versus HOCl. In addition to mediating protein modification and protein aggregation [39-41], HOCl's damaging effect is also due to its ability to oxidatively destroy the catalytic sites, in this case the heme group, of the enzymes to different degrees. The severity of this process depends mainly on the HOCl level in the solution milieu. Collectively, increased MPO/CAT ratio may lead to accumulation of free iron, in addition to modifications of the structure and function of proteins that are biologically relevant to the initiation and maintenance of inflammation. On the other hand, increasing CAT:MPO ratio, scavenging HOCl/H_2O_2, and/or inhibiting MPO minimize instant HOCl-mediated damage, thereby keeping both enzymes catalytically active. Understanding the mechanism of the mutual influences of individual enzyme activities should be useful for elucidating disease mechanisms and developing clinically useful therapy.

Catalase modulates the toxicity of the MPO-H_2O_2 system

The toxicity of the MPO/HOCl system could be controlled by multiple factors including CAT and other related enzymes that catalyze the conversion of H_2O_2 to H_2O, HOCl scavengers, and MPO inhibitors (e.g. taurine, methionine, and melatonin). Disturbance of this balance may play a crucial role in catalytic function of both MPO and CAT. HOCl may influence MPO/CAT steady-state catalysis by acting either as a heme ligand [14], or as a mediator of heme destruction [14, 15], protein modification [40, 41], and protein aggregation [39]. The interaction of OCl^-

with both MPO and CAT includes its ability to form the Fe(III)-OCl complex and subsequently generate compounds I and II [14, 39, 42]. Moreover, the ability of both MPO-Fe(III)-OCl and CAT-Fe(III)-OCl complexes to decay to higher oxidative states, namely Compounds I and II, was also illustrated by diode array stopped-flow measurements [14, 42-44].

A model showing competition between MPO and CAT for H_2O_2 and resulting HOCl-mediated heme destruction and/or modification

HOCl is a potent oxidant that under normal physiological conditions operates as an antimicrobial agent [14, 39, 43, 44]. However, under pathological conditions, the rate of HOCl production becomes excessive, and the compound may mediate protein modification and/or hemoprotein heme destruction leading to the generation of free iron [14, 39, 43, 44]. A kinetic model that describes the interaction of HOCl with MPO/CAT intermediates is shown in Fig. 7. Under normal conditions, CAT catalyzes the conversion of H_2O_2 to H_2O, whereas MPO employs Cl^- in the presence of H_2O_2 to generate HOCl through the formation of MPO Compound I (ferryl porphyrin π cation radical, Fe(IV)=O(+π•)). The partitioning of H_2O_2 consumption between the two pathways depends mainly on two enzymes' concentration and their relative affinity to H_2O_2. Rapid kinetic measurements have shown that the reaction between CAT and H_2O_2 followed a second order reaction with a rate constant of 5.0×10^6 s^{-1} M^{-1} [45]. In parallel, the second order rate constant for the reaction of MPO−Fe(III) with H_2O_2 to form MPO Compound I was found to be 1.8×10^7 M^{-1} s^{-1} at 25ºC, whereas the second order rate constant of Compound I with Cl^- to form HOCl was found to be 4.7×10^6 M^{-1} s^{-1} [46, 47]. HOCl, when accumulated in the MPO/CAT solution mixture, competes with H_2O_2 for the catalytic sites of both enzymes and binds to the heme iron at a near diffusion rate forming the corresponding Fe(III)-OCl complexes.

Fe(III)-OCl complexes then decay to Compound II through the formation of Compound I. Compound II is formed through the reaction of HOCl with CAT-Fe(III)/MPO-Fe(III) and is the prime target of HOCl-mediated heme destruction and subsequent free iron release [14-17]. Alternatively, CAT Compound I reacts with H_2O_2 to complete its natural catalytic cycle that could partially protect against HOCl-mediated heme destruction but not against protein aggregation [48]. Consistent with this notion, Bonini *et al.* have demonstrated that the reaction of CAT with HOCl leads to protein oxidation both in vitro and in cell cultures, which is a primary event leading to CAT aggregation and consequent loss of function [39]. Taking into consideration that heme groups in the catalase molecule are deeply buried [49], Krych-Madej and Gebicka have shown that the sulfur-containing amino acid residues on the protein surface are the main targets for HOCl modification [42]. Irreversible CAT heme damage was detected by the authors when the enzyme was treated with higher concentrations of HOCl [42]. Regardless of enzymatic inactivation through either heme destruction, protein aggregation, or a combination of both, HOCl mediates the inhibition of both enzymes [39, 42, 50]. The degree of CAT/MPO inactivation is significantly higher at lower CAT: MPO ratios because only a small portion of total enzymes is estimated to remain active after sequential addition of H_2O_2.

With H_2O_2 serving as a limiting substrate of HOCl synthesis under such circumstances, modulation in the ratio between both enzymes provides significant protection against HOCl insults. This protection is due to the increase in H_2O_2-consumption by CAT and CAT compound I, increase in the heme content provided by MPO and CAT, or a combination of both. This process is reflected by the shift to the right in the MPO inactivation curve (Fig. 2A). In marked contrast, increasing the MPO/CAT ratio could lead to more HOCl production, but the MPO inactivation curve still shifts to the right (Fig. 2B), indicating that the HOCl/heme content ratio

plays a crucial role in hemoprotein inhibition, with H_2O_2 serving to limit HOCl synthesis under such circumstances. MPO contains two heme prosthetic groups while CAT is a tetramer with four heme groups, which allows the CAT heme groups to be more susceptible to HOCl attacks [51, 52]. However, the ability of CAT Compound I to utilize H_2O_2 as a substrate could favor protein modification and enzyme inactivation rather than CAT heme destruction, especially at low HOCl concentrations, consistent with other reports [39, 42]. The loss of catalytic activity of both enzymes by HOCl mediated heme destruction and/or protein modification is also supported by evaluating different MPO inactivation curves that display the same heme content (e.g., 2:1 CAT:MPO (Fig 2A) and 3:1 MPO:CAT (Fig. 2B) but display different degrees of protection. Our UV-visible studies showed that higher H_2O_2 concentration was required to inactivate a correspondingly higher concentration of MPO and CAT (Fig. 3A), a process that could be prevented in the presence of MPO inhibitors and/or HOCl scavengers. Taken together, the ability of HOCl to mediate MPO/CAT inhibition through multiple pathways may provide new insight into biological roles, particularly in organs that experience exposure to wide variations of MPO/CAT levels. The prevention of free iron generation by catalase suppresses reactions between metals and highly reactive free radicals such as the hydroxyl radical (•OH) produced through the Fenton reaction, which are extremely damaging to tissues and further perpetuate the inflammatory environment [53, 54].

The crosstalk between MPO and CAT

CAT is an enzymatic antioxidant, which under normal conditions, serves a signaling function and plays an important role in protecting the cell from oxidative damage mediated by reactive oxygen species (ROS) [55, 56]. H_2O_2 is freely diffusible through membranes, and under

pathological conditions, such as during atherosclerosis, asthma, and other inflammatory processes, rates of H_2O_2 production become excessive. CAT deficiency or its inactivation by HOCl plays an important role in up-regulating the catalytic activity of MPO that occurs at sites of inflammation. CAT and MPO crosstalk with each other through the diffusion of H_2O_2 and HOCl, whether the enzymes are in different locations or are co-localized in the same cell [57]. In our current work, we have shown that HOCl, the final product of MPO, promotes CAT inactivation and MPO heme destruction resulting in the release of oxidants. Taken together, our findings support a biologically relevant mechanism, which delineates a novel role for MPO in the pathogenesis of inflammatory diseases.

Alterations of MPO/CAT ratio in respiratory and cardiovascular pathologies

Specific interrelationships between variants of CAT and MPO and their role in the etiology of respiratory conditions related to oxidative stress have been infrequently explored. Significantly increased concentrations of plasma MPO, nitric oxide metabolites, and iron were detected in asthmatic individuals [58]. It is believed that the loss of SOD and CAT activities is related to oxidative modifications, while other antioxidant gene polymorphisms are linked to the susceptibility to develop asthma. In support of this, CAT activity was lower in the red blood cells of asthmatic children and in bronchoalveolar lavage specimens of asthmatic lungs, as compared to healthy individuals [59]. In addition to asthma, the crosstalk between CAT and MPO also plays an important role in cystic fibrosis (CF). Although there are no significant differences in the concentrations or activities of H_2O_2 in breath condensate of CF patients compared to healthy individuals, sputum specimens from CF patients were found to contain large amounts of active MPO (5.93±4.8 μM) and CAT (0.31±0.18 μM) [1]. Epithelial lining fluid and plasma in CF

patients were characterized by a marked deficiency of non-enzymatic antioxidants such as GSH, taurine, and methionine compared to controls [1, 60]. An abnormal accumulation of total iron, and iron-regulatory cytokines such as interleukin-1 beta and tumor necrosis factor alpha were found in the sputum of the patients with chronic bronchitis and, to a greater extent, in patients with CF [61, 62]. The involvement of HOCl in mediating the generation of free iron through heme destruction in disease pathology might highlight its involvement in the production of •OH in the presence of H_2O_2. Accumulation of free iron may promote oxidative injury and enhance bacterial growth [63]. Additionally, overload of free iron is also associated with many inflammatory disorders such as arthritis, diabetes, psychiatric illness, and liver disease, and is also a risk factor for cancer and heart diseases [4, 5, 7, 64, 65].

Furthermore, MPO and its oxidant products, which predict increased cardiovascular risk, have been implicated in the pathogenesis of atherosclerosis [66]. MPO mediates lipid peroxidation and conversion of LDL to an atherogenic form [66]. Recently, it has been shown that MPO-derived oxidants can also modify HDL, causing an impairment of cholesterol efflux [66]. Thus, MPO has been considered a useful biomarker for cardiovascular disease [67]. CAT, among other antioxidant enzymes, has been shown to protect the endothelium of human aorta against apoptosis associated with oxidized forms of LDL [68]. Such results highlighted the key role of H_2O_2 in atherogenesis as well as CAT and other related antioxidant enzymes' role in insulating against oxidative stress of the cardiovascular system by impacting H_2O_2-mediated signaling and HOCl-production [69, 70]. Therefore, blocking the MPO/HOCl chlorination machinery might be a useful therapeutic approach in vascular diseases and other inflammatory conditions, where iron overload is a significant etiopathological factor.

Conclusion

In conclusion, it is conjectured that CAT and MPO crosstalk with each other, independent of their localization, through the diffusion of the substrate (H_2O_2) and product (HOCl) in a common pro-oxidant mechanism. Therefore, HOCl overproduction, which occurs in many inflammatory diseases, could lead to the imbalance of MPO and CAT through protein modification and/or heme destruction. Inactivation of both enzymes leads to H_2O_2 accumulation, which, when combined with free iron, causes further oxidative damage to the host by generation of •OH. Thus, we describe a HOCl-based regulatory mechanism that enables hemoprotein instant release of free iron and protein modification that can be attenuated by CAT. This may provide new insights into biological roles for MPO and CAT, particularly in organs that experience elevated MPO/HOCl levels. The crosstalk between MPO and CAT is remarkable and suggests that there are biological roles for their interaction which remain to be considered. These are under current investigation in our laboratories

Acknowledgements: Dr. Iyad Ali was a Fulbright Senior Research Scholar in Dr. Abu-Soud laboratory.

Figure legends

Figure 1. MPO inactivation by self-generated HOCl and its prevention by CAT. (A) A typical recording by an H_2O_2-selective electrode demonstrating the dramatic MPO feedback inhibition mediated by self-generated HOCl after incremental consecutive addition of H_2O_2 (10 µ M, 3 µL in 3 ml reaction mixture) (denoted by the arrows) to a continuously stirred phosphate buffer (200 mM, pH 7.4) containing 40 nM MPO and 100 mM Cl^-, at 25° C. Identical experiment performed (B) By replacing MPO/Cl^- with 40 nM CAT. (C) In the presence of combination of 40 nm MPO, 40 nM CAT and 100 mM Cl^-. The data shown are representative of three independent experiments.

Figure 2. Area under the curves of the H_2O_2 selective-electrode traces reflecting the maximum signal intensity to time of H_2O_2 consumption by both MPO and CAT, were determined and plotted as a function of H_2O_2 added to the same solution mixture. A) When an incremental H_2O_2 solution (10 µM, 3 µL) was added to a continuously stirred phosphate buffer (200 mM, pH 7.4) supplemented with fixed amount of MPO (40 nM) and increasing concentration of CAT (0, 40, 50,120, and 160 nm) in the presence of fixed amount of Cl^- (100) mM, at 25° C. B) When an incremental H_2O_2 solution (10 µM, 3 µL) was added to a continuously stirred phosphate buffer (200 mM, pH 7.4) supplemented with fixed amount of CAT (40 nM) and increasing concentration of MPO (0, 40, 50,120, and 160 nm) in the presence of fixed amount of Cl^- (100) mM, at 25ºC. The data shown are representative of three independent experiments.

Figure 3. Melatonin inhibits the MPO chlorinating activity and prevents MPO/CAT inactivation mediated by HOCl. (A) A typical recording by an H_2O_2-selective electrode demonstrating the dramatic effect of CAT in protecting MPO from HOCl insults after incremental consecutive addition of equal amounts of H_2O_2 (10 µM, 3 µL in 3 ml reaction mixture) (denoted by the

arrows) to a continuously stirred phosphate buffer (200 mM, pH 7.4) containing 40 nM MPO, 40 nM CAT, and 100 mM Cl^-, at 25° C. (B) An identical experiment performed in the presence of 100 µM melatonin. The data shown are representative of three independent experiments.

Figure 4. Absorbance spectra of MPO (0.6 µM) (dashed line), CAT (0.6 µM) (dotted line), and combination of both (sold line) recorded in 200 mM phosphate buffer, (pH 7.4) from 350-700 nm, at 25ºC. The data shown are representative of three independent experiments.

Figure 5. Effects of methionine in inhibiting MPO/CAT heme destruction mediated by MPO-generated HOCl. A cuvette containing phosphate buffer supplemented with CAT (~0.6 µM) and 100 mM Cl^- was mixed well and used to zero the absorbance reading from 300-500 nm (dotted line), at 25ºC. The dashed line is the prominent Soret absorbance centered at 430 nm after adding of MPO (~0.6 µM) to the solution mixture, followed by sequential incremental addition of H_2O_2 (20 µM, 3 µl) to the solution mixture. The spectra were recorded immediately after each H_2O_2 addition (A). Spectra collected 10 min. after each H_2O_2 addition (B). The same experiments in the presence of methionine (100 µM) (C). The data shown are representative of three independent experiments.

Figure 6. The susceptibility of MPO versus CAT heme destruction by HOCl. A typical recording by an H_2O_2-selective electrode when three consecutive addition of exogenous HOCl (10 µM)/H_2O_2 (10 µM) to a continuously stirred phosphate buffer (200 mM, pH 7.4) supplemented with 40 nM MPO and 40 nM CAT at 25° C to insure MPO/CAT inactivation. The reaction mixture then received 100 mM Cl^- followed by two consecutive H_2O_2 additions (denoted by the arrows). The data shown are representative of three independent experiments.

Figure 7. Working kinetic model for the H_2O_2 and HOCl interaction with both MPO and CAT.

References

[1] D. Worlitzsch, G. Herberth, M. Ulrich, G. Doring, Eur Respir J, vol. 11, 1998, pp. 377-383.

[2] S.A. Comhair, S.C. Erzurum, Antioxid Redox Signal, vol. 12, 2010, pp. 93-124.

[3] G.M. Saed, M.P. Diamond, N.M. Fletcher, Gynecol Oncol, vol. 145, 2017, pp. 595-602.

[4] W.Y. Ong, B. Halliwell, Ann N Y Acad Sci, vol. 1012, 2004, pp. 51-64.

[5] D. Trinder, C. Fox, G. Vautier, J.K. Olynyk, Gut, vol. 51, 2002, pp. 290-295.

[6] S. Defrere, J.C. Lousse, R. Gonzalez-Ramos, S. Colette, J. Donnez, A. Van Langendonckt, Mol Hum Reprod, vol. 14, 2008, pp. 377-385.

[7] L.Y. Chau, Proc Natl Sci Counc Repub China B, vol. 24, 2000, pp. 151-155.

[8] K. Yamaguchi, M. Mandai, S. Toyokuni, J. Hamanishi, T. Higuchi, K. Takakura, S. Fujii, Clin Cancer Res, vol. 14, 2008, pp. 32-40.

[9] N.M. Fletcher, Z. Jiang, R. Ali-Fehmi, N.K. Levin, J. Belotte, M.A. Tainsky, M.P. Diamond, H.M. Abu-Soud, G.M. Saed, Cancer Biomark, vol. 10, 2011, pp. 267-275.

[10] G. Bartosz, Reactions, Processes, Springer, Berlin, 2005, pp. 109-149.

[11] S.J. Klebanoff, J Leukoc Biol, vol. 77, 2005, pp. 598-625.

[12] M.J. Davies, C.L. Hawkins, D.I. Pattison, M.D. Rees, Antioxid Redox Signal, vol. 10, 2008, pp. 1199-1234.

[13] E.A. Podrez, H.M. Abu-Soud, S.L. Hazen, Free Radic Biol Med, vol. 28, 2000, pp. 1717-1725.

[14] D. Maitra, F. Shaeib, I. Abdulhamid, R.M. Abdulridha, G.M. Saed, M.P. Diamond, S. Pennathur, H.M. Abu-Soud, Free Radic Biol Med, vol. 63, 2013, pp. 90-98.

[15] O.I. Aruoma, B. Halliwell, Biochem J, vol. 248, 1987, pp. 973-976.

[16] C.E. Souza, D. Maitra, G.M. Saed, M.P. Diamond, A.A. Moura, S. Pennathur, H.M. Abu-Soud, PLoS One, vol. 6, 2011, pp. e27641.

[17] D. Maitra, J. Byun, P.R. Andreana, I. Abdulhamid, M.P. Diamond, G.M. Saed, S. Pennathur, H.M. Abu-Soud, Free Radic Biol Med, vol. 51, 2011, pp. 374-386.

[18] H.M. Abu-Soud, D. Maitra, J. Byun, C.E.A. Souza, J. Banerjee, G.M. Saed, M.P. Diamond, P.R. Andreana, S. Pennathur, Free Radic Biol Med, vol. 52, 2012, pp. 616-625.

[19] I. Sliskovic, I. Abdulhamid, M. Sharma, H.M. Abu-Soud, Free Radic Biol Med, vol. 47, 2009, pp. 1005-1013.

[20] T. Lu, S. Galijasevic, I. Abdulhamid, H.M. Abu-Soud, Br J Pharmacol, vol. 154, 2008, pp. 1308-1317.

[21] S. Galijasevic, I. Abdulhamid, H.M. Abu-Soud, Biochemistry, vol. 47, 2008, pp. 2668-2677.

[22] S. Galijasevic, I. Abdulhamid, H.M. Abu-Soud, Free Radic Biol Med, vol. 44, 2008, pp. 1570-1577.

[23] D.R. Ramos, M. Victoria Garcia, L.M. Canle, J. Arturo Santaballa, P.G. Furtmuller, C. Obinger, Arch Biochem Biophys, vol. 466, 2007, pp. 221-233.

[24] A.J. Kettle, L.P. Candaeis, Redox Rep, vol. 5, 2000, pp. 179-184.

[25] M. Banerjee, N. Banerjee, P. Ghosh, J.K. Das, S. Basu, A.K. Sarkar, J.C. States, A.K. Giri, Toxicol Appl Pharmacol, vol. 249, 2010, pp. 47-54.

[26] I.V. Gorudko, V.A. Kostevich, A.V. Sokolov, E.V. Shamova, I.V. Buko, E.E. Konstantinova, V.B. Vasiliev, S.N. Cherenkevich, O.M. Panasenko, Bull Exp Biol Med, vol. 154, 2012, pp. 23-26.

[27] V.L. Kinnula, M. Myllarniemi, Antioxid Redox Signal, vol. 10, 2008, pp. 727-738.

[28] S. Ghosh, A.J. Janocha, M.A. Aronica, S. Swaidani, S.A. Comhair, W. Xu, L. Zheng, S. Kaveti, M. Kinter, S.L. Hazen, S.C. Erzurum, J Immunol, vol. 176, 2006, pp. 5587-5597.

[29] G. Proteasa, Y.R. Tahboub, S. Galijasevic, F.M. Raushel, H.M. Abu-Soud, Biochemistry, vol. 46, 2007, pp. 398-405.

[30] R.M. Rakita, B.R. Michel, H. Rosen, Biochemistry, vol. 29, 1990, pp. 1075-1080.

[31] Y.R. Tahboub, S. Galijasevic, M.P. Diamond, H.M. Abu-Soud, J Biol Chem, vol. 280, 2005, pp. 26129-26136.

[32] P.C. Andrews, N.I. Krinsky, J Biol Chem, vol. 257, 1982, pp. 13240-13245.

[33] J. Vlasits, C. Jakopitsch, M. Schwanninger, P. Holubar, C. Obinger, FEBS Lett, vol. 581, 2007, pp. 320-324.

[34] L. Wang, M. Bassiri, R. Najafi, K. Najafi, J. Yang, B. Khosrovi, W. Hwong, E. Barati, B. Belisle, C. Celeri, M.C. Robson, J Burns Wounds, vol. 6, 2007, pp. e5.

[35] S.D. Feng Y, Bolton JR, J. Environ. Eng. Sci, 2007, pp. 277-284.

[36] C. Storkey, M.J. Davies, D.I. Pattison, Free Radic Biol Med, vol. 73, 2014, pp. 60-66.

[37] S.M. Dellegar, S.A. Murphy, A.E. Bourne, J.C. DiCesare, G.H. Purser, Biochem Biophys Res Commun, vol. 257, 1999, pp. 431-439.

[38] L.K. Folkes, L.P. Candeias, P. Wardman, Arch Biochem Biophys, vol. 323, 1995, pp. 120-126.

[39] M.G. Bonini, A.G. Siraki, B.S. Atanassov, R.P. Mason, Free Radic Biol Med, vol. 42, 2007, pp. 530-540.

[40] D.I. Pattison, M.J. Davies, Biochemistry, vol. 45, 2006, pp. 8152-8162.

[41] D.I. Pattison, M.J. Davies, Biochemistry, vol. 44, 2005, pp. 7378-7387.

[42] J. Krych-Madej, L. Gebicka, Int J Biol Macromol, vol. 80, 2015, pp. 162-169.

[43] P.G. Furtmuller, C. Obinger, Y. Hsuanyu, H.B. Dunford, Eur J Biochem, vol. 267, 2000, pp. 5858-5864.

[44] R. Floris, R. Wever, Eur J Biochem, vol. 207, 1992, pp. 697-702.

[45] A. Deisseroth, A.L. Dounce, Physiol Rev, vol. 50, 1970, pp. 319-375.

[46] L.A. Marquez, H.B. Dunford, J Biol Chem, vol. 270, 1995, pp. 30434-30440.

[47] L.A. Marquez, J.T. Huang, H.B. Dunford, Biochemistry, vol. 33, 1994, pp. 1447-1454.

[48] B. Chance, H. Sies, A. Boveris, Physiol Rev, vol. 59, 1979, pp. 527-605.

[49] M.R. Murthy, T.J. Reid, 3rd, A. Sicignano, N. Tanaka, M.G. Rossmann, J Mol Biol, vol. 152, 1981, pp. 465-499.

[50] D. Maitra, F. Shaeib, I. Abdulhamid, R.M. Abdulridha, G.M. Saed, M.P. Diamond, S. Pennathur, H.M. Abu-Soud, Free Radic Biol Med, vol. 63, 2013, pp. 90-98.

[51] T.J. Reid, 3rd, M.R. Murthy, A. Sicignano, N. Tanaka, W.D. Musick, M.G. Rossmann, Proc Natl Acad Sci U S A, vol. 78, 1981, pp. 4767-4771.

[52] T.J. Fiedler, C.A. Davey, R.E. Fenna, J Biol Chem, vol. 275, 2000, pp. 11964-11971.

[53] C.C. Winterbourn, Toxicol Lett, vol. 82-83, 1995, pp. 969-974.

[54] J. Prousek, Pure and Applied Chemistry, vol. 79, 2009, pp. 2325-2338.

[55] R. Dringen, P.G. Pawlowski, J. Hirrlinger, J Neurosci Res, vol. 79, 2005, pp. 157-165.

[56] H.B. Dunford, Heme peroxidases, John Wiley, New York, 1999, pp. 1-507.

[57] G. Bauer, J Inorg Biochem, vol. 179, 2018, pp. 10-23.

[58] O.B. Ekmekci, O. Donma, E. Sardogan, N. Yildirim, O. Uysal, H. Demirel, T. Demir, Biochemistry (Mosc), vol. 69, 2004, pp. 462-467.

[59] A.M. Fitzpatrick, D.P. Jones, L.A. Brown, Antioxid Redox Signal, vol. 17, 2012, pp. 375-408.

[60] J.H. Roum, R. Buhl, N.G. McElvaney, Z. Borok, R.G. Crystal, J Appl Physiol (1985), vol. 75, 1993, pp. 2419-2424.

[61] N.R. Henig, M.R. Tonelli, M.V. Pier, J.L. Burns, M.L. Aitken, Thorax, vol. 56, 2001, pp. 306-311.

[62] D.W. Reid, Q.T. Lam, H. Schneider, E.H. Walters, Eur Respir J, vol. 24, 2004, pp. 286-291.

[63] K. Hantke, Curr Opin Microbiol, vol. 4, 2001, pp. 172-177.

[64] H. Ohshima, M. Tatemichi, T. Sawa, Arch Biochem Biophys, vol. 417, 2003, pp. 3-11.

[65] J. Schiller, B. Fuchs, J. Arnhold, K. Arnold, Curr Med Chem, vol. 10, 2003, pp. 2123-2145.

[66] S.J. Nicholls, S.L. Hazen, J Lipid Res, vol. 50 Suppl, 2009, pp. S346-351.

[67] M.L. Brennan, S.L. Hazen, Curr Opin Lipidol, vol. 14, 2003, pp. 353-359.

[68] S.J. Lin, S.K. Shyue, M.C. Shih, T.H. Chu, Y.H. Chen, H.H. Ku, J.W. Chen, K.B. Tam, Y.L. Chen, Atherosclerosis, vol. 190, 2007, pp. 124-134.

[69] M.R.F. Linton, P.G. Yancey, S.S. Davies, W.G. Jerome, E.F. Linton, W.L. Song, A.C. Doran, K.C. Vickers, in: K.R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, K. Dungan, A. Grossman, J.M. Hershman, G. Kaltsas,

C. Koch, P. Kopp, M. Korbonits, R. McLachlan, J.E. Morley, M. New, L. Perreault, J. Purnell, R. Rebar, F. Singer, D.L. Trence, A. Vinik, D.P. Wilson (Eds.), Endotext, South Dartmouth (MA), 2000.

[70] A. Abdal Dayem, M.K. Hossain, S.B. Lee, K. Kim, S.K. Saha, G.M. Yang, H.Y. Choi, S.G. Cho, Int J Mol Sci, vol. 18, 2017.

Figure 1

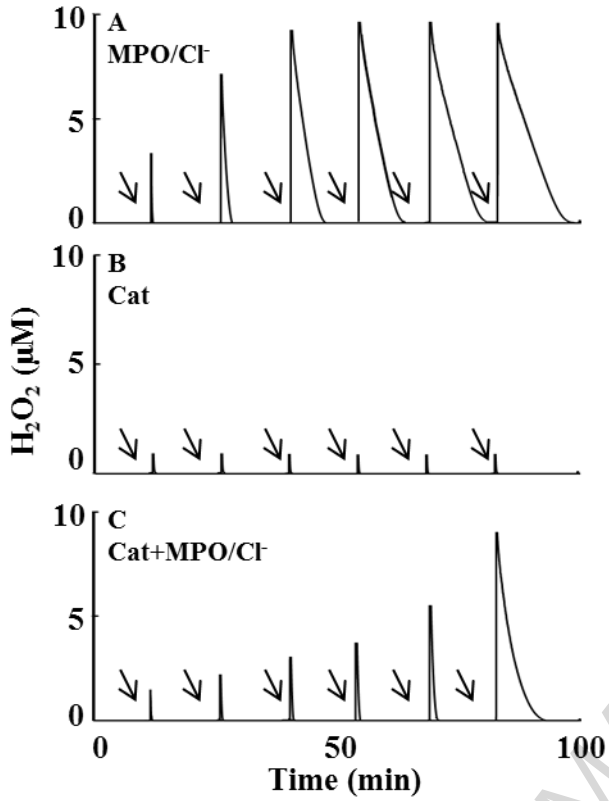


Figure 2

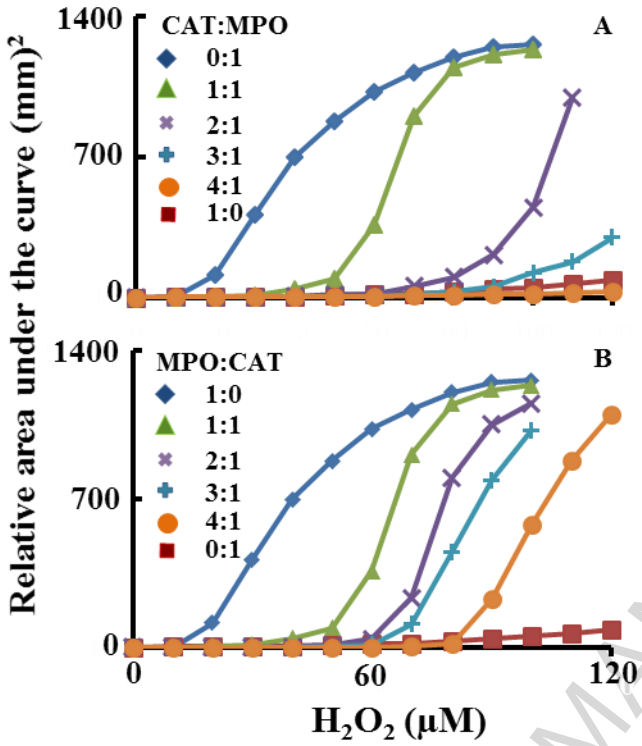


Figure 3

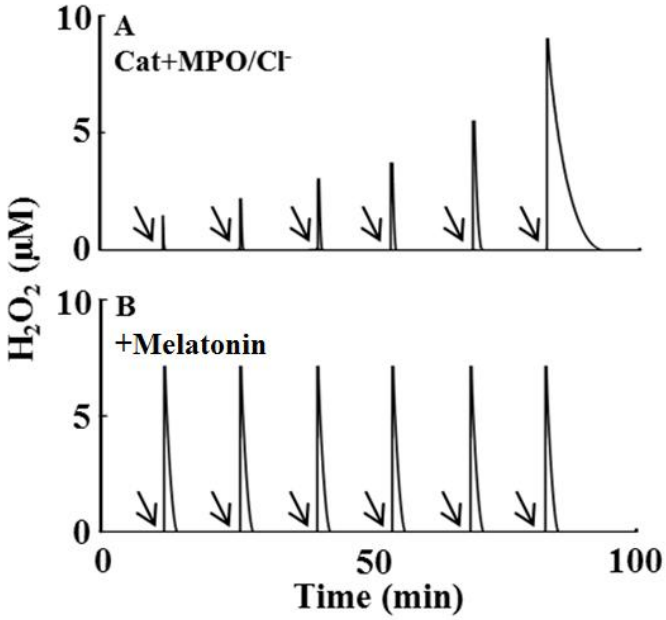


Figure 4

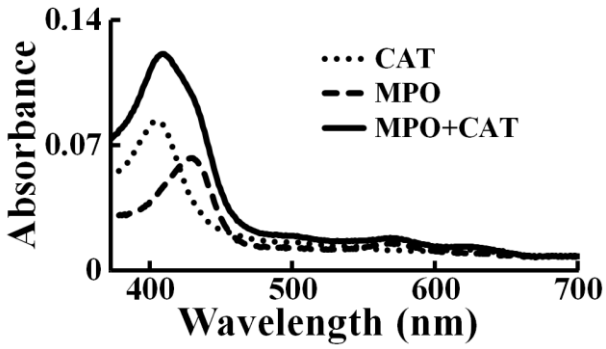


Figure 5

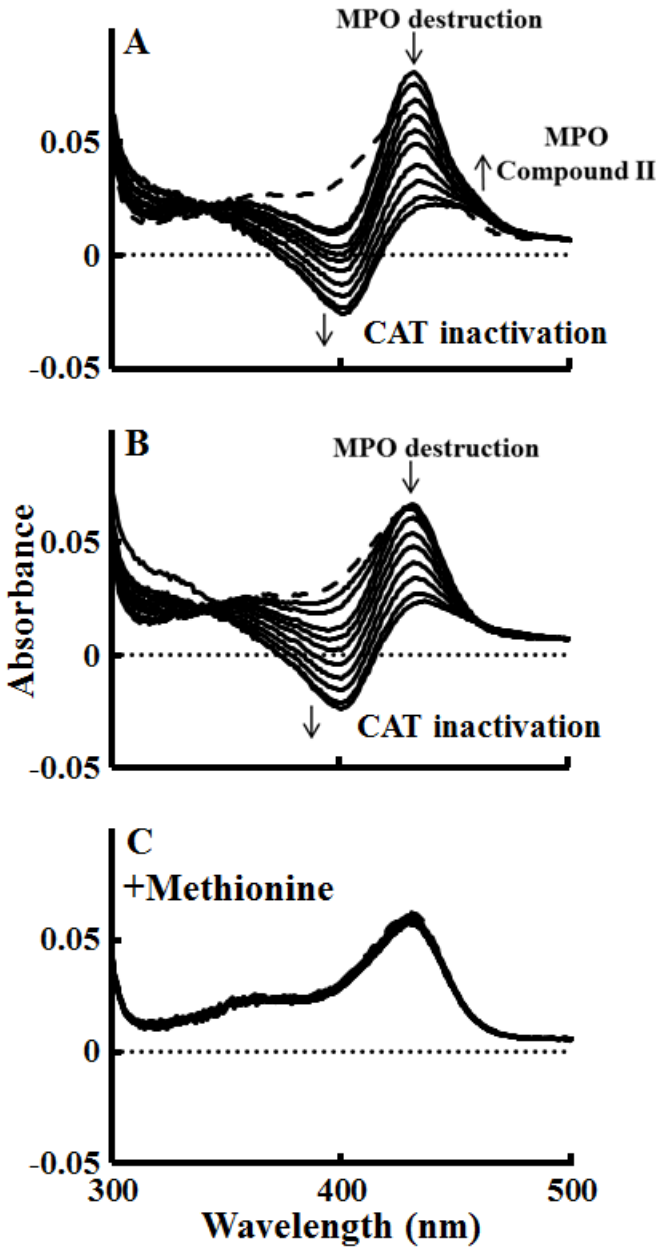


Figure 6

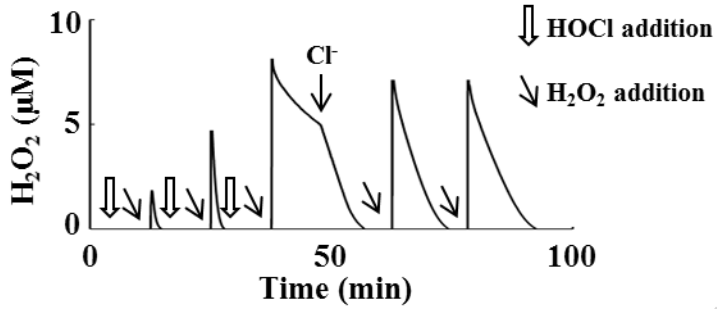
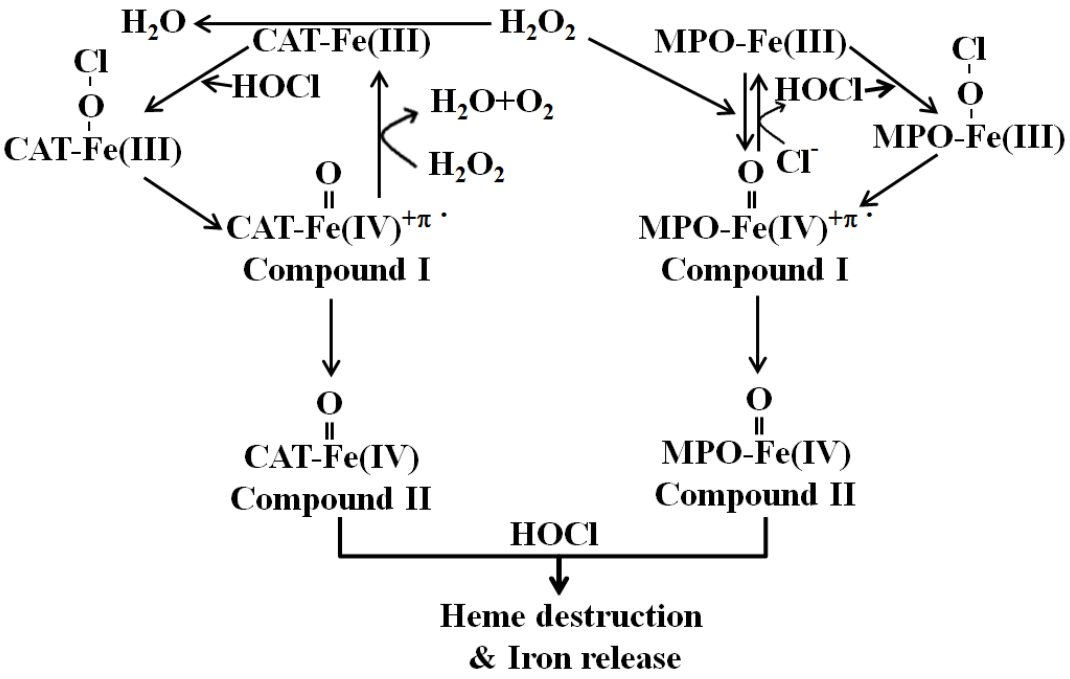
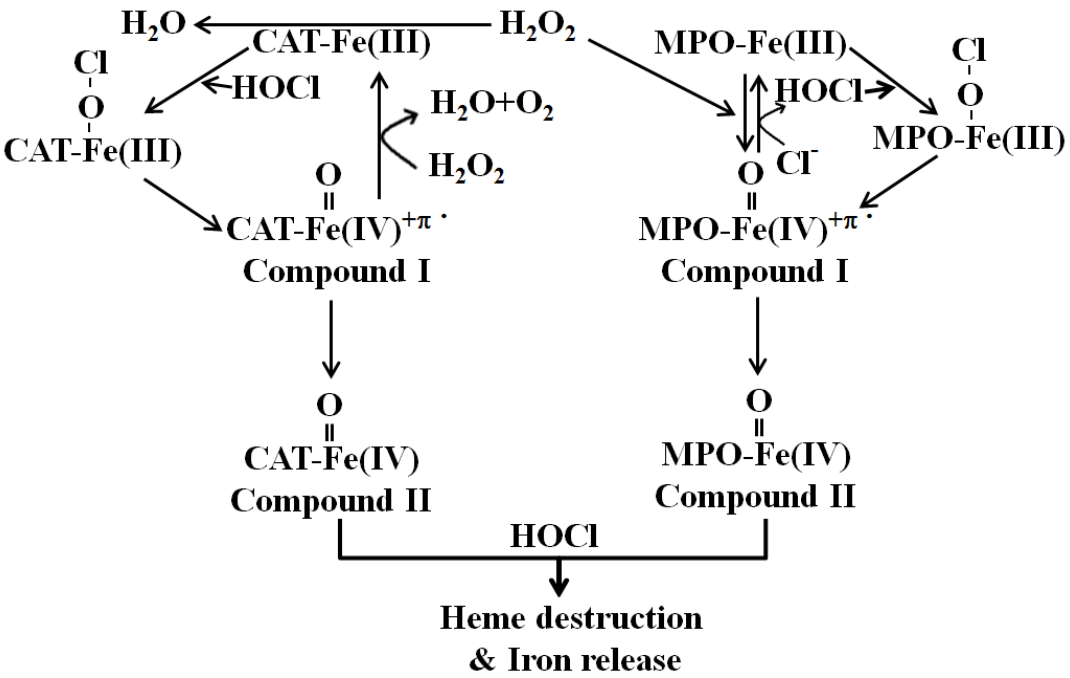


Figure 7



Graphical Abstract

Synopsis: Working kinetic model for the H_2O_2 and HOCl interaction with both MPO and CAT.



Highlights

- Hypochlorous acid (HOCl) generated by myeloperoxidase (MPO) destroys hemoprotein heme moiety.

- Catalase (CAT) not only competes with MPO for hydrogen peroxide ($H_2O_{2)}$ but also scavenges HOCl

- CAT protective role vs. HOCl damaging effect depends on MPO/CAT ratio

- Altered MPO/CAT ratio regulates HOCl production and ceases heme destruction

- Modulation of MPO/CAT activities may be a fundamental feature of catalysis