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"Peroxidases in Biology and Medicine"

Heme-Peroxidase Biochemistry: Pulling Surprises From Their Pockets

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INTRODUCTION

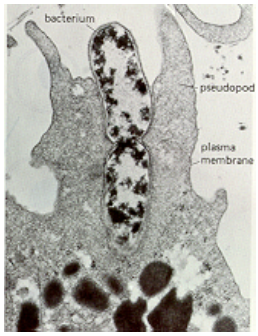
Since their discovery peroxidases have given scientists many wonderful surprises. From the beginning, their ability to generate colourful products made them ideal enzymes to study. Their own distinct absorption spectra provided confirmation of the existence of enzyme-substrate intermediates. But they do not quite fit the definition of an enzyme. Specificity is not their forte. These guys oxidize a host of substrates and take promiscuity to a completely new level. One of the big surprises was that myeloperoxidase oxidizes chloride to hypochlorous acid (HOCl). Its closley related cousin, eosinophil peroxidase, struggles to snatch electrons from chloride but has no problems with bromide and produces hypobromous acid (HOBr). Even more surprising is that these toxic bleaches are actually produced in our bodies and are intimately involved in fighting infections and wreaking havoc during inflammation. A major revelation for me was discovering that superoxide is a physiological substrate of myeloperoxidase that modulates production of chlorine bleach. From that time I have been enamoured with myeloperoxidase. I have worked with Christine Winterbourn at the Christchurch School of Medicine since 1986 to understand the enzymology of this fascinating green protein and to demonstrate a role for chlorine bleach in inflammatory diseases. In this lecture I would like to introduce you to the captivating world of mammalian peroxidases. My focus will be mainly on myeloperoxidase but other important peroxidases include eosinophil peroxidase and prostaglandin synthase.

PEROXIDASES AND WHITE BLOOD CELLS

Neutrophils and eosinophils are packed full with peroxidases. Neutrophils and eosinophils are white blood cells that form part of our primary host defense system. Neutrophils ingest bacteria into phagocytic vacuoles where they are killed by oxidants and antibiotic peptides. Myeloperoxidase is the most abundant protein in neutrophils, is also present in monocytes, and is expressed in microglia. Eosinophils are richly endowed with eosinophil peroxidase. These granulocytic cells are a major source of oxidants in our bodies. They generate vast amounts of oxidants to kill

Leger's impression of a neutrophil fighting infection and promoting inflammation.

invading micro-oganisms. The importance of oxidants in host defence is demonstrated by an experiment of nature in which these cells are unable to generate superoxide. People with this condition, known as chronic granulomatous disease, suffer from severe and often fatal infections.



A neutrophil phagocytosing a bacterium. (From Molecular Biology of the Cell 2nd Edn)

Neutrophils and eosinophils are also responsible for promoting tissue damage in inflammatory conditions. These include rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, chronic obstructive pulmonary disease, sepsis, and asthma. The current challenge is to show that oxidants play a substantive role in inflammation. This is best achieved by understanding how leukocytes produce oxidants, what these oxidants are, and how they react with biological molecules.

HISTORY: FASHION VS SCIENCE

Stimulated neutrophils undergo a burst of respiration in which oxygen is reduced to superoxide. About 40 years ago Sbarra and Karnovsky demonstrated that neutrophils undergo a marked respiratory burst when they phagocytoze bacteria. This was cyanide insensitive, so not part of mitochondrial respiration. It was shown that oxygen was converted to hydrogen peroxide. At that stage it was thought that hydrogen peroxide was responsible for killing bacteria. Then Klebanoff showed that neutrophils use myeloperoxidase to oxidize chloride to hypochlorous acid (HOCl). HOCl is a highly toxic oxidant that kills almost all pathogens. The story seemed complete. Neutrophils were reducing oxygen to hydrogen peroxide, which was used by the peroxidase to produce the ultimate anti-microbial agent chlorine bleach.

We use chlorine bleach inside and outside our bodies to kill germs.

However, bleach was largely ignored as fashion took control of science. Just after McCord and Fridovich discovered superoxide dismutase, Babior demonstrated that superoxide is the primary product of the neutrophil respiratory burst. He and others, notably Segal, showed that the cells contain an NADPH-oxidase that reduces molecular oxygen to superoxide. Superoxide was the molecule of fascination at the time and it was believed that it was the bactericidal product of neutrophils. Then came a period of distraction. Also the case for myeloperoxidase and bleach was not helped because myeloperoxidase-deficiency was found to be relatively common (1:3000) and generally not to have major consequences. However, some individuals, particularly those with diabetes often get severe infections.

Superoxide was found to be poor at killing bacteria. Its toxicity was then explained in terms of its ability to drive the Fenton reaction and produce hydroxyl radicals. Much effort was put into demonstrating that neutrophils could produce hydroxyl radicals. Indeed many studies showed that hydroxyl radicals were formed but most indicated that hydroxyl radical production was tiny and involved myeloperoxidase in some way. A situation developed where oxidants were trying to be imposed on the neutrophil. Scientific fashion was determining what was the chief oxidant of neutrophils. Although hydroxyl radicals and superoxide were receiving a huge amount of attention because of their general importance in biology, they are not major players as far as toxic neutrophil oxidants are concerned. A more reflective approach was required to appreciate Nature's design for the purposeful generation of oxidants.

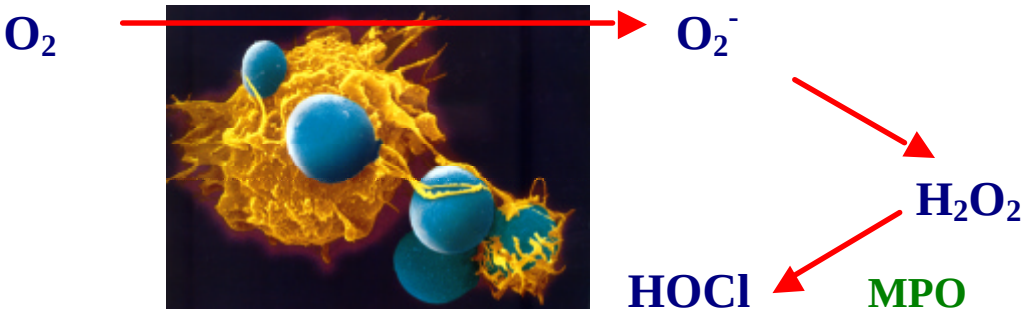
How super is superoxide?

If only researchers had stopped and wondered why neutrophils have a green hue!

MPO gives pus, phlegm and bogeys their greenish tinge.

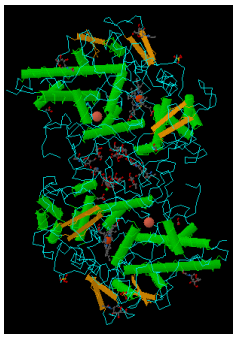
When Weiss showed that 25% of extracellular hydrogen peroxide was converted to HOCl it became clear that chlorine bleach is a major oxidant generated by neutrophils. Subsequently, Hurst showed that at least 10% of the oxygen consumed by neutrophils was converted to HOCl that reacted inside phagosomes. These two results clearly confirmed Klebanoff's earlier assertion that myeloperoxidase plays a central part in neutrophil oxidant production.

The following sequence of reactions is now accepted to occur when a neutrophil is stimulated either by ingesting bacteria or by a soluble stimulus such as phorbol esters, formylated peptides, and cytokines.



When neutrophils ingest bacteria and fungi they convert oxygen to hypochlorous acid.

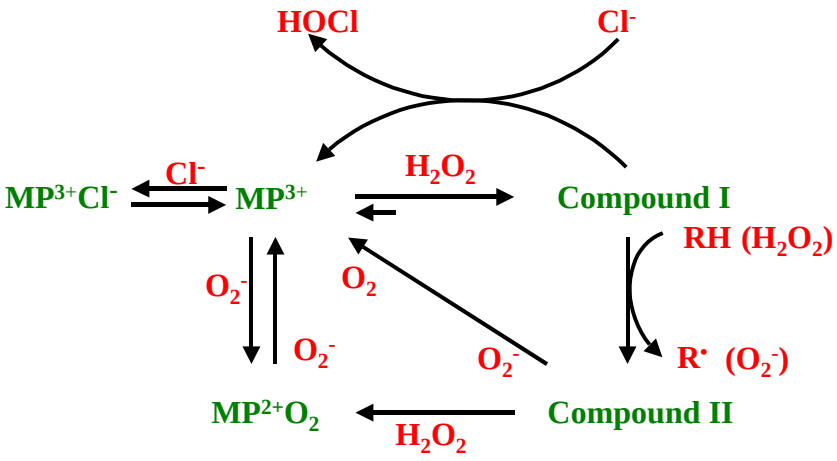
MYELOPEROXIDASE



Myeloperoxidse is a heme enzyme

Myeloperoxidse is a fascinating green enzyme. The protein is a tetramer consisting of two identical dimers each containing a heme prosthetic group. The heme groups act independently and have equal reactivity with hydrogen peroxide and reducing substrates. Myeloperoxidase undergoes a complex array of redox transformations that enable it to produce HOCl, degrade hydrogen peroxide to oxygen and water, convert tyrosine and other phenols and anilines to reactive free radicals, and hydroxylate aromatic substrates via a cytochrome P450 type activity. It also reacts rapidly with superoxide in reactions that modulate production of HOCl.

The native ferric enzyme reacts with H_2O_2 to form the redox intermediate compound I, which has a formal oxidation state of 5+. It has a two-electron reduction potential of 1.16V; i.e. it is strongly oxidizing. Under physiological conditions, Cl^- and thiocyanate (SCN^-) are equally preferred substrates for compound I. They reduce it back to the ferric enzyme, and HOCl or HOSCN are formed, respectively. This sequence of reactions is termed the halogenation cycle. Compound I can also react with numerous organic substrates, such as tyrosine, via transfer of a single electron to produce a free radical. In the process the enzyme is converted to compound II, which has a formal oxidation state of 4+. Compound II reacts with a second organic substrate molecule to produce another free radical and complete the peroxidation cycle. Poor peroxidase substrates react with compound I and trap the enzyme as compound II. Under these conditions production of HOCl is inhibited. However, superoxide overcomes this inhibition by reducing compound II back to the active enzyme. In this way, superoxide boosts production of HOCl. Myeloperoxidase also has catalase activity; i.e. hydrogen peroxide reacts with both the ferric enzyme and compound I to liberate oxygen and water. Oxymyeloperoxidase ($MP^{2+}O_2$), or compound III, is formed from the reaction of superoxide with native enzyme. It is the predominant form of the enzyme in stimulated neutrophils and is involved in hydroxylation of phenols.



The reaction mechanism of myeloperoxidase. MP^{3+} is the native enzyme; RH is an organic substrate such as tyrosine.

The complexities of the reactions of myeloperoxidase lend themselves to kinetic modelling.

Modelling tells you what is wrong!

Kinetic modelling of the reactions of myeloperoxidase is a useful approach to understanding what activities of the enzyme are biologically relevant. It has revealed that some previous assumptions of how the enzyme acts are wrong. For example, not all of its reactions with hydrogen peroxide have been appreciated. At low concentrations of chloride, myeloperoxidase is a very good catalase. Modelling predicts that most of the hydrogen peroxide formed inside phagosomes should be converted to HOCl. Also, reactions of superoxide with compound II and compound III are essential for intraphagosomal production of HOCl.

There has been a renaissance in peroxidase research. When Heinecke and Hazen showed that myeloperoxidase-dependent oxidation of tyrosine promoted peroxidation of LDL, they rekindled interest in peroxidases. They also demonstrated that myeloperoxidase was present in atherosclerotic lesions, as was 3-chlorotyrosine, which is a specific biomarker of HOCl. Stocker and colleagues provided strong evidence that HOCl-modified proteins were present in atherosclerotic lesions. All these researchers established that myeloperoxidase can convert LDL to a form that is readily taken up by macrophages. This work provided compelling evidence that myeloperoxidase is a candidate for initiating atherosclerosis. It also focussed attention on the possible involvement of myeloperoxidase in numerous inflammatory diseases. In particular, myeloperoxidase and eosinophil peroxidase may be responsible for causing tissue damage in respiratory diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease. The peroxidases accumulate in the airways of patients with these diseases and produce both HOCl and HOBr.

MYELOPEROXIDASE-DERIVED OXIDANTS

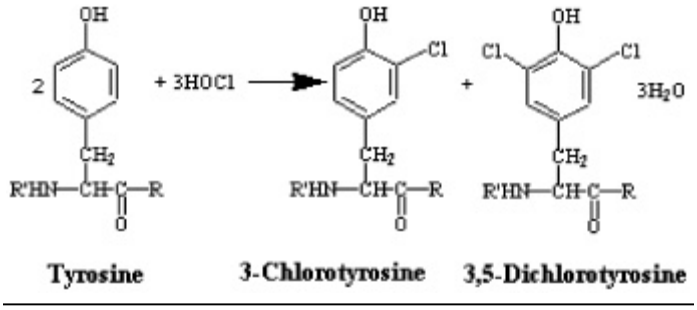
Hypochlorous acid is a potent oxidant. HOCl has much potential to inflict tissue destruction at sites of inflammation. At physiologically plausible doses it is toxic to all cell types, reacting rapidly with a myriad of biological molecules. It inactivates enzymes, readily cross-links proteins, and depletes cells of glutathione, ATP, and ascorbate. Its reactivity with biomolecules is intermediate between hydroxyl radical and peroxynitrite. Thiols, along with methionine residues, are about 100 times more reactive with HOCl than are most other biological molecules. Chloramines (and bromamines) are secondary products of the reaction of HOCl (or HOBr) with amines. They are less reactive but retain the oxidising capability of their precursors. HOBr has been less studied, but appears similar in reactivity to HOCl. HOSCN is a much milder oxidant and in contrast to the others, does not react

HOCl makes globin (left) aggregate (right).

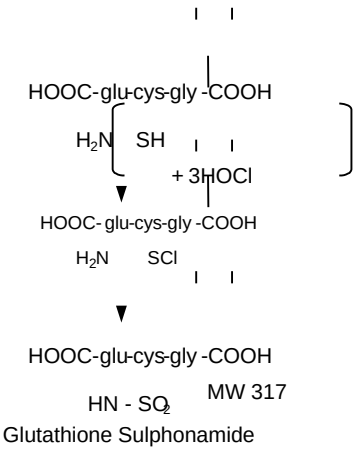
with methionine, ascorbate or amines. Therefore, it should be longer lived and have even higher thiol selectivity. Halogenation of biomolecules, particularly nucleic acids, may have important physiological consequences. It has been suggested that cancers associated with chronic inflammation may result from chlorination reactions of HOCl.

BIOMARKERS OF HYPOCHLOROUS ACID

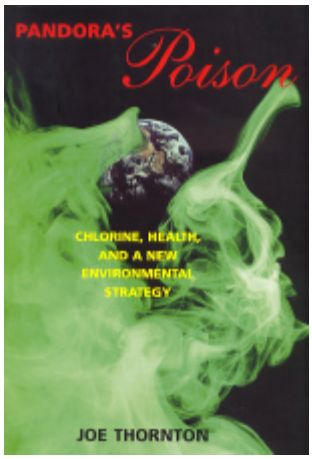
Biomarkers of hypohalous acids hold the key to establishing a role for peroxidases in inflammation. The high reactivity of HOCl precludes its direct measurement in the body. Therefore, specific and stable products of its reactions with biomolecules are needed to show that HOCl has been produced at sites of infection and inflammation. MPO promotes the chlorination of tyrosyl residues in proteins to produce 3-chlorotyrosine and 3,5-dichlorotyrosine. These can be detected by hydrolyzing proteins and derivatizing the resulting amino acids so that they are amenable to analysis by gas chromatography with mass spectrometry. These biomarkers have provided clear evidence that HOCl is produced in atherosclerotic lesions, in the airways of children with cystic fibrosis, and in the lungs of asthmatic.



Winterbourn has demonstrated that HOCl reacts with glutathione to produce several oxidation products including glutathione sulphonamide. It is the fastest reaction of HOCl with any biological molecule and the sulphonamide appears to be specific to HOCl. Glutathione sulphonamide has enormous potential as a biomarker because it will be a major physiological product of HOCl. Carbonyls are formed on proteins when they are oxidized by a variety of oxidants, including HOCl. Measurement of protein carbonyls is useful because it gives an estimate of total oxidative stress. However, protein carbonyls are not specific to a particular oxidant so they should be measured in combination with more specific biomarkers such as chlorinated tyrosines. The appropriate use of these sensitive biomarker assays should establish whether peroxidases contribute to a particular pathology. Currently, they are the best way to demonstrate a role for oxidants in disease processes.



CONCLUSIONS



Chlorine produced inside & outside our bodies is an important poison.

In recent times there have been many new advances in the chemistry and biology of peroxidases. I am sure many more surprises will be pulled from their heme pockets. Our future understanding of these complex enzymes will be derived from developments in accurate screening methods for peroxidase deficiencies, linkage of polymorphisms to disease, and the use of suitable biomarkers and specific inhibitors to define their physiological activity. Do not be surprised if HOCl or HOBr are found to be more of a threat to our health than the nasty chlorinated compounds we pollute our environment with.

RECOMMENDED READING

1. Klebanoff SJ. Phagocytic cells: products of oxygen metabolism. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: basic principles and clinical correlates, 1 ed. New York: Raven Press Ltd, 1988:

2. Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase and bacterial killing. **Blood** 1998; **92**: 3007-3017.

3. Kettle AJ, Winterbourn CC. Myeloperoxidase: A key regulator of neutrophil oxidant production. **Redox Report** 1997; **3**: 3-15.

4. Winterbourn CC, Kettle AJ. Biomarkers of myeloperoxidase-derived hypochlorous acid. **Free Radical Biol.Med.** 2000; **29**: 403-409.

5. Winterbourn CC, Vissers MCM, Kettle AJ. Myeloperoxidase. **Curr.Opin.Hematol.** 2000; **7**: 53-58.

6. Heinecke JW. Mechanisms of oxidative damage by myeloperoxidase in atherosclerosis and other inflammatory disorders. **J Lab.Clin.Med.** 1999; **133**: 321-325.

7. Mitra SN, Slungaard A, Hazen SL. Role of eosinophil peroxidase in the origins of protein oxidation in asthma. **Redox.Rep.** 2000; **5**: 215-224.

8. Nauseef WM. Quality control in the endoplasmic reticulum: lessons from hereditary myeloperoxidase deficiency. **J Lab.Clin.Med.** 1999; **134**: 215-221.