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## **Physical-Chemical Characterization of Myeloperoxidase (MPO) and Related Degradation Products**

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# Physical-Chemical Characterization of Myeloperoxidase (MPO) and Related Degradation Products

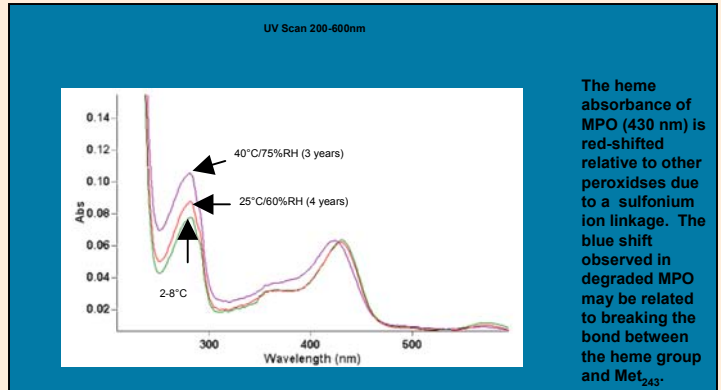


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## ABSTRACT

The goal of this study was to render porcine MPO a well-characterized biopharmaceutical via physical-chemical characterization. MPO was purified to near homogeneity, formulated, and evaluated for stability using reversed phase HPLC (RP-HPLC), UV spectrophotometry (UV), size exclusion chromatography (SEC), Refractive Index (RI) detection, Laser Light Scattering (LLS) detection, and Mass Spectrometry (MS) and an enzyme activity assay using guaiacol as the substrate. Sequencing of porcine DNA was performed as an aid in peptide map identification by LC/MS/MS. Intact MPO was found to be too heterogeneous for direct mass spectrometric characterization. MPO was first denatured, reduced, and blocked then separated into its respective heavy and light subunits by RP-HPLC followed by tryptic mapping. The light chain analyzed by LC/MS, had a m/z value of approximately 12,600. Deglycosylation of the heavy subunit was achieved using three successive enzymatic digestion steps with neuraminidase, O-glycosidase, and glycopeptidase-F, in order to remove sialic acid, O- and N-linked carbohydrates prior to LC/MS. Stability studies on intact MPO revealed that more than 75% of the protein activity, and RP-HPLC peak area were retained after storage at 40°C for up to 1 year. SEC with LLS detection showed no differences between samples stored at 2-8°C, 25°C/60% RH, or 40°C/75% RH; however, the UV-VIS spectrum of samples of MPO stored at 40°C/75% RH showed a significant shift in the secondary maximum. These results indicate that MPO is remarkably stable and that its quaternary structure is consistent with published data.

## Characterization of MPO by UV, DSC, and RP-HPLC



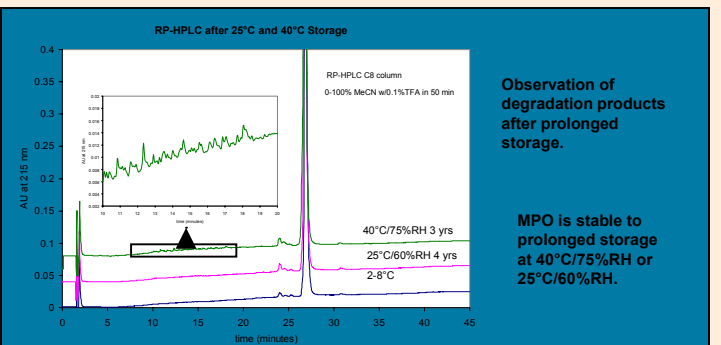
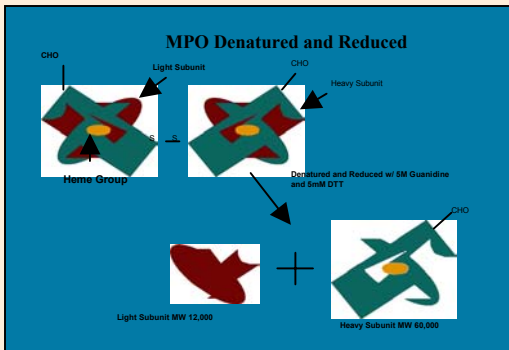
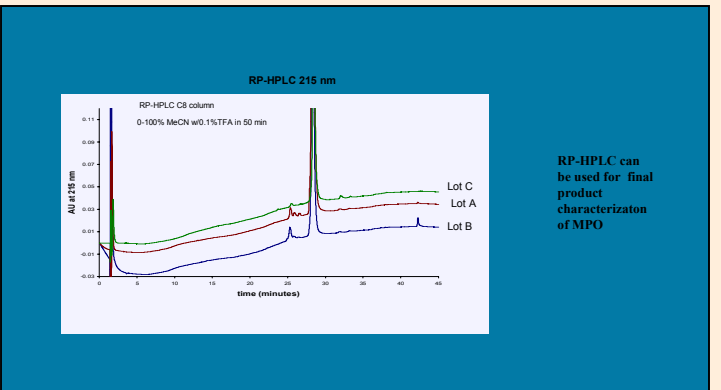
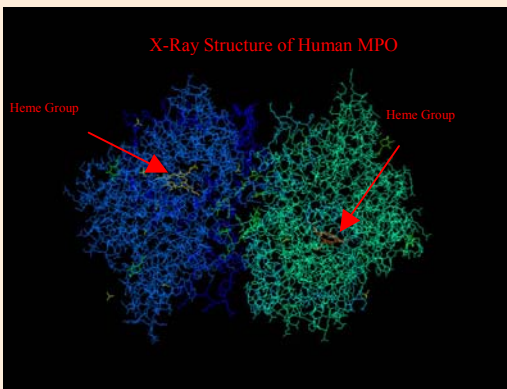
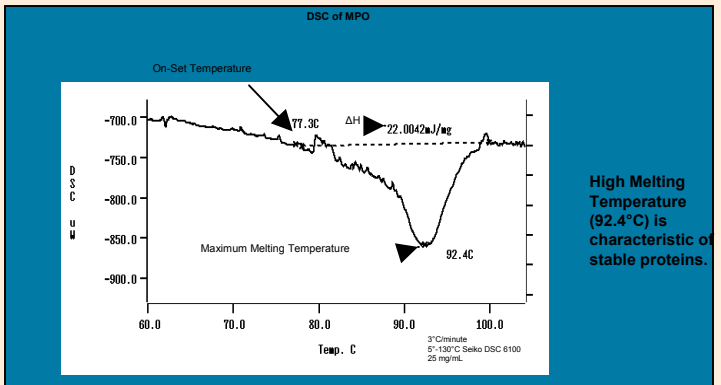
**What is Myeloperoxidase?**  
**What does it do? How does it do it?**

MPO is a heme containing protein found in granules in neutrophils and monocytes.

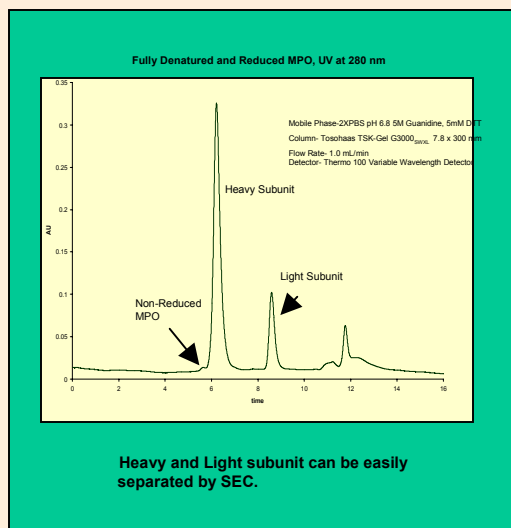
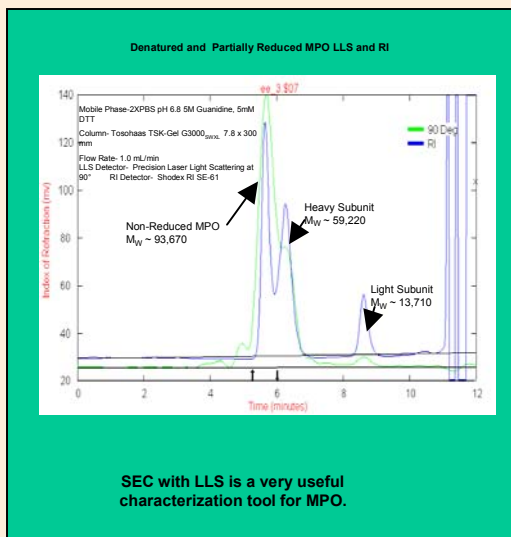
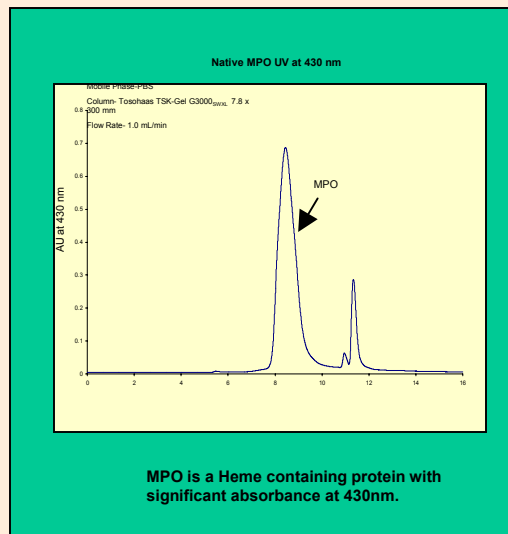
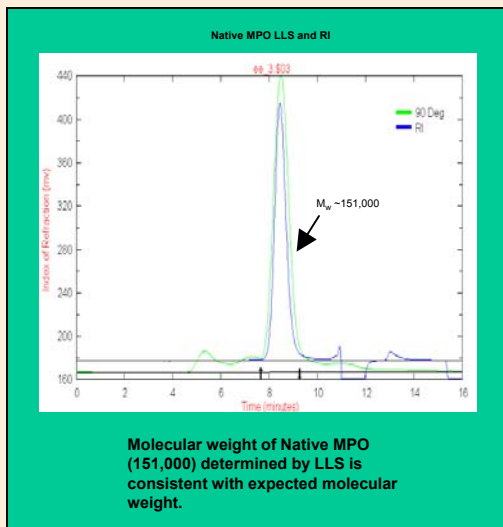
MPO is an enzyme with potent antimicrobial and tumoricidal activity.

MPO catalyzes the production of hypochlorous acid. This is BLEACH!!!!!!

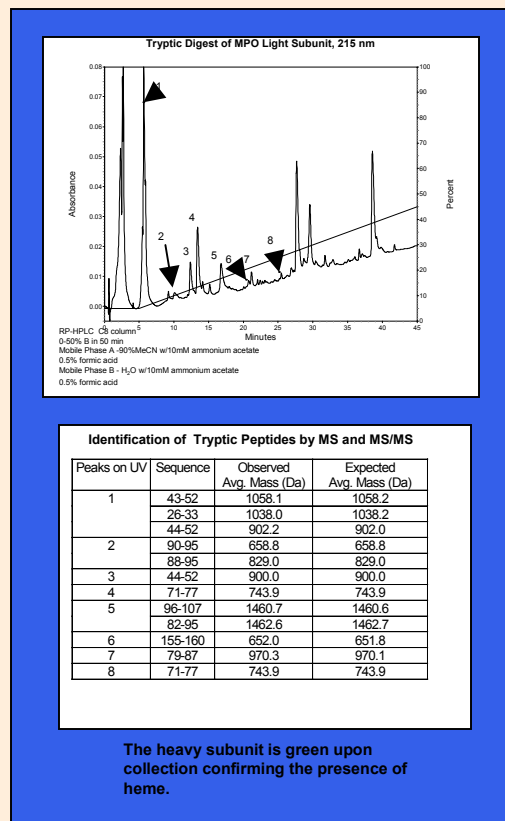
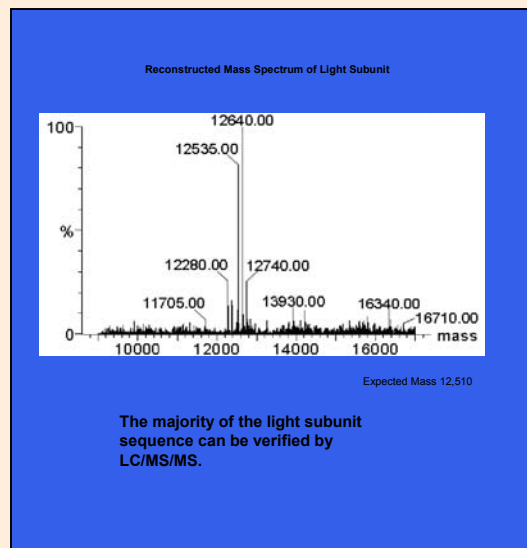
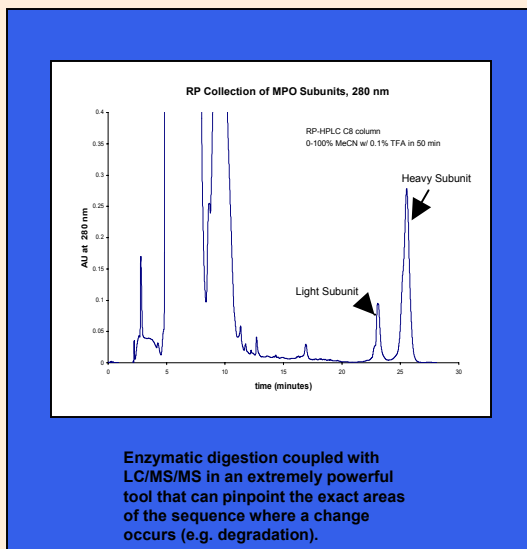
The reaction catalyzed by MPO is:

$$\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}_3\text{O}^+ \rightarrow \text{HOCl} + 2\text{H}_2\text{O}$$


## Characterization of size and mass of MPO by SEC with UV, RI and LLS



## Characterization of MPO subunits by MS, and tryptic digest by LC/MS/MS



### Summary/Conclusion

- The heavy and light subunits of Porcine MPO are easily resolved by SEC and RP-HPLC. The heavy subunit contains covalently bound heme, as expected.
- MPO is unusually stable to prolonged storage at up to 40°C and also to thermal denaturation. DSC shows that the thermal transition for MPO occurs around 90°C, an unusually high temperature for any protein.
- Degradation of MPO upon storage is accompanied by a blue shift in the heme absorption spectrum. This represents a change in the environment surrounding the heme.
- Intact MPO gives a complex mass spectrum. After the subunits have been separated the light subunit gives well resolved MS peaks and the subunit can be fragmented into tryptic peptides which are amenable to MS. The heavy subunit requires deglycosylation and separation of tryptic peptides in order to return an interpretable signal. Interpretation of the MS data was facilitated by sequencing of pDNA to provide protein sequence information.
- Porcine MPO has been characterized by SEC using UV detection, LLS and RI. Results show that the molecule has the MW expected for an A<sub>2</sub>B<sub>2</sub> tetramer in solution, as expected. Porcine MPO has little tendency to aggregate as judged by SEC-LLS.
- Results of physical chemical characterization of Porcine MPO are consistent with expected structures based on published historical data.
- Further work on the characterization of the heavy subunit and its carbohydrate moiety are on-going
- SDS-PAGE and bioactivity data (not shown here) are consistent with these conclusions.