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Effect of unsaturation in fatty acids on the binding and oxidation by myeloperoxidase: Ramifications for the initiation of atherosclerosis

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ABSTRACT

Oxidation of low density lipoproteins (LDL) in the presence of myeloperoxidase and subsequent uptake of the oxidized LDL by specialized receptors on macrophages has been suggested as an initiating event of atherosclerosis. Oxidized fatty acid chains within the glycerophospholipids of LDL have been implicated as the recognition feature by the receptors. The ability of three fatty acids (oleic, linoleic, and arachidonic acids) typically contained in the lipid portion of the glycerophospholipids to bind and be oxidized by myeloperoxidase was measured by spectroscopically observing interactions of the lipids with the heme prosthetic group of the enzyme. As unsaturation increases in the lipid chain, myeloperoxidase binds and oxidizes the fatty acid more readily, as measured by K_D, K_M, and k_{cat}. A possible mechanism of the free radical oxidation by myeloperoxidase is discussed.

Oxidation of low density lipoproteins (LDL) has been implicated in the initiation and progression of atherosclerosis,[1–3] with the preferential binding of oxidized LDL (oxLDL) to specialized CD36 receptors on macrophages.[4,5] Upon binding to the receptors and subsequent uptake of the oxLDL into the arterial cell wall, the lipoprotein releases its sequestered cholesterol. This release results in the formation of lipid-laden 'foam cells,' the foundation of arterial plaques. An overriding feature of this inflammatory event and subsequent hardening of the deposits is the presence of oxidized lipids from LDL. While many endogenous enzymes, including lipoxygenases, cyclooxygenases, and cytochrome P450 mono-oxygenases are capable of oxidizing lipids,[6,7] the presence in the plaques of myeloperoxidase (MPO), a heme containing enzyme secreted by neutrophils in response to inflammation, suggests its role in the oxidation of LDL.[8] More importantly, clinical studies have indicated increased levels of MPO in patients with coronary artery disease.[9,10] Oxidized apolipoprotein B, the protein component of LDL, was thought to be primarily involved in the recognition event of oxLDL to macrophages, but recently this hypothesis has been questioned.[11,12] Instead, in vitro and in vivo studies have indicated MPO initiates lipid oxidation prior to protein oxidation, and that lipid modification is at least partially responsible for the recognition and uptake of the LDL into macrophages, rather than fusion due to the protein component of LDL.[5,11]

Neutrophils produce hydrogen peroxide and discharge MPO in response to inflammation; reduction of hydrogen peroxide by MPO results in the concomitant oxidation of endogenous halogens or thiocyanate to generate hypohalous acid or hypothiocyanous acid oxidants, respectively.[13] In vivo, these strong reactive oxidants provide the first line attack against invading pathogens; however, hypochlorous and hypothiocyanous acid are also capable of oxidizing lipids and protein.[14] Moreover, nitrogen containing species have also been implicated as mediators of lipid oxidation in a MPO–hydrogen peroxide system.[4] A relatively unexplored alternative pathway for the oxidation of LDL, however, is the direct oxidation of lipids by MPO in the presence of hydrogen peroxide without an additional oxidizable substrate. While both of these paths can generate oxidized lipids, much of the research to this point has focused on the oxidizing power of activated small molecules such as chlorides or thiocyanate on lipids, as these compounds are thought to provide the common mechanism by which all mammalian peroxidases target pathogens and other biomolecules. Of interest, then, is the largely ignored but physiologically relevant alternate pathway of lipid oxidation through direct oxidation of the lipid substrate by MPO.

To oxidize lipids directly, MPO first needs to interact with the lipids. MPO has been shown to bind to the LDL complex,[15] but no research has shown binding of a specific component of the glycerophospholipids. Because evidence suggests that the oxidized fatty acid chain on a glycerophospholipid provides a recognition factor for binding to macrophages,[5] the propensity of different fatty acid side chains found in the LDL complex to bind and be oxidized by MPO would provide insight into the mechanism of LDL oxidation, particularly in the absence of other oxidants. Previous spectroscopic experiments have shown that some heme proteins are

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capable of binding to fatty acids, with polyunsaturated lipids binding more readily than saturated or monounsaturated lipids.[16–18] Moreover, the rate of oxidation by MPO after binding to the respective fatty acids can also be measured spectroscopically, either by colorimetrically measuring the concentration of oxidized lipids after a set amount of time or by monitoring changes in the iron oxidation state of the heme prosthetic group in MPO.[19] This latter method is based on the catalytic cycle of the plant and animal peroxidases, in which the native peroxidase's heme prosthetic group is in an Fe(III) oxidation state. Once exposed to hydrogen peroxide, the iron is oxidized in a two-electron step to Fe(IV)=O (oxo-ferryl) porphyrin cation radical (compound **I**) while hydrogen peroxide is reduced, as shown below in the peroxidase catalytic cycle (Eq. 1).

$$\text{Heme-Fe(III)} + H_2O_2 \rightarrow \text{Heme}^{\cdot}\text{-Fe(IV)=O} + H_2O \quad (1)$$

$$\text{Heme}^{\cdot}\text{-Fe(IV)=O} + RH \rightarrow \text{Heme-Fe(IV)=O} + R^{\cdot} \quad (2)$$

$$\text{Heme-Fe(IV)=O} + RH \rightarrow \text{Heme-Fe(III)} + R^{\cdot} + H_2O \quad (3)$$

Reduction of compound I back to MPO's native state can occur either by a two-electron oxidation of a small molecule such as a halide, or compound I can undergo the peroxidase cycle in which two one-electron reduction steps initially form compound II (Fe(IV)=O, with the porphyrin radical reduced to neutral porphyrin, Eq. 2) and then the native enzyme (Fe(III), Eq. 3). In the process, two equivalents of substrate (RH) are oxidized to free radicals through the loss of an electron.[20,21]

The more standard colorimetric method of determining rates of oxidation of lipids typically relies on measurements of a predetermined lipid oxidative product, such as malondialdehyde or conjugated diene, at a set time after initiation of the peroxidase catalyzed reaction. The variety of oxidative products formed from unsaturated fatty acids, however, can complicate the analysis of kinetic measurements. Moreover, conjugated diene measurements can only be made on fatty acids capable of forming a conjugated system, such as linoleic and arachidonic acids. To avoid these potential difficulties in determining oxidation rates, kinetics in this Letter were measured by observing changes in the iron oxidation state of the heme prosthetic group. A method originally used to measure the kinetics of MPO oxidation of indole derivatives was adapted for determining the initial rate of oxidation of fatty acids by MPO.[19] Human neutrophil MPO (Athens Research Technology, Athens, GA) was used for all experiments; fatty acids (Sigma, St. Louis, MO) were used without further purification. The spectroscopic data from both binding experiments and kinetics measurements was used to calculate K_D, K_M, and k_{cat} values for three major fatty acids found in the glycerophospholipid component of LDL (Fig. 1), oleic (**1**), linoleic (**2**), and arachidonic acid (**3**), to analyze the relationship between the structure of the fatty acid chains in LDL and the ability to bind to MPO and then oxidize in the presence of the enzyme and hydrogen peroxide.

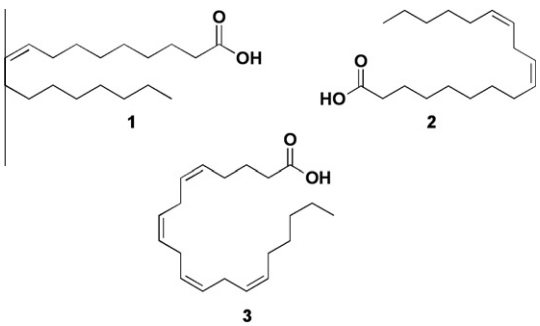

Figure 1. Structures of major fatty acids (oleic acid (1), linoleic acid (2), and arachidonic acid (3)) found in LDL glycerophospholipids.

MPO was shown spectroscopically to bind all three fatty acid chains found in LDL glycerophospholipids, with an increasing affinity of the fatty acid for MPO as the degree of unsaturation in the lipid increased as measured by K_D (Table 1). The change in the Soret region of the visible spectrum of MPO upon titration with unsaturated fatty acid indicates specific binding of the lipids near the heme prosthetic group. Dissociation constants (K_D) for each MPO–lipid complex were determined as previously described.[22] In a typical experiment, MPO (3 μM, determined using the molar extinction coefficient, $\varepsilon_{430} = 91{,}000\ M^{-1}\ cm^{-1}$) was titrated with aliquots of the appropriate lipid previously dissolved in buffer (0.10 M phosphate, pH 7.4) containing 1% (w/v) Tween 20 to increase solubility. After each lipid addition, the mixture was allowed to stand for 1 min, and the resulting spectrum was scanned from 300 to 800 nm. A plot of the differential absorbance was made by subtracting each successive scan with lipid added from the scan with MPO only (Fig. 2). The absorption maxima and minima from the differential spectrum, A_{max} and A_{min}, were subtracted to yield $\Delta A_{max-min}$. A_{max} was 456 nm for all lipids tested; A_{min} was 416 nm for both oleic and linoleic acids and 422 nm for arachidonic acid. Dissociation values were determined by fitting the curve of $\Delta A_{max-min}$ versus final lipid concentration (10–100 μM) with GraphPad Prism software, using either a hyperbolic curve fit or Hill fit (Fig. 3).

MPO was also shown spectroscopically to oxidize all three fatty acids found in the glycerophospholipids of LDL. Taking advantage of the substantial differences in absorption maxima between native MPO, compound **I**, and compound **II**, accurate spectroscopic measurements of one-electron oxidation of lipids can be acquired. Compound **II** is the most stable intermediate for the MPO peroxidase cycle, and thus kinetic measurements were determined spectroscopically by measuring the change in absorbance of compound II as the iron atom was reduced to its ferric state in native MPO in the presence of lipid.[19] In a typical experiment, a solution of the enzyme (2 μM) in buffer (0.1 M phosphate buffer, pH 7.4) was mixed with a five fold excess of hydrogen peroxide (10 μM final concentration). The resulting solution was monitored spectroscopically for the formation of the stable oxidized form (compound **II**) of myeloperoxidase indicated by an absorption maxima at 456 nm (Fig. 4).[19] Immediately after evidence of compound II formation, lipid solution was added to yield final concentrations from 0.0625 to 1.0 mM and the progress of the oxidation reaction was monitored for 3 min over the wavelength range of 300–700 nm. Initial rates of oxidation, υ_o, at each lipid concentration were determined from difference spectrum curves made by subtracting each consecutive spectrum from the original compound II spectrum (Fig. 5). Plotting the change in absorbance, $\Delta A_{max-min}$ (A_{max} is 456 nm and A_{min} is 418 nm for all lipids), obtained from the difference spectrum curves, versus the time in seconds gave initial rates of oxidation for each concentration of lipid used. Initial rates of oxidation were linear for 1–2 min after initiation, depending upon lipid concentration, and this linear portion was used for rate determinations. Subsequent plotting of initial rates of oxidation versus final lipid concentration yielded K_M, k_{cat}, and the specificity constant k_{cat}/K_M from the Michaelis–Menton hyperbolic curve after analysis with GraphPad Prism software (Fig. 6).

Table 1
Binding and kinetic parameters for MPO with fatty acids[a]

Lipid	K_D (μM)	K_M (μM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}\ s^{-1}$)
Oleic acid	52	35	0.082	2.3×10^3
Linoleic acid	24	49	0.160	3.3×10^3
Arachidonic acid	16	151	0.170	1.13×10^3

[a] Results are an average of a minimum of three independent trials for each lipid.

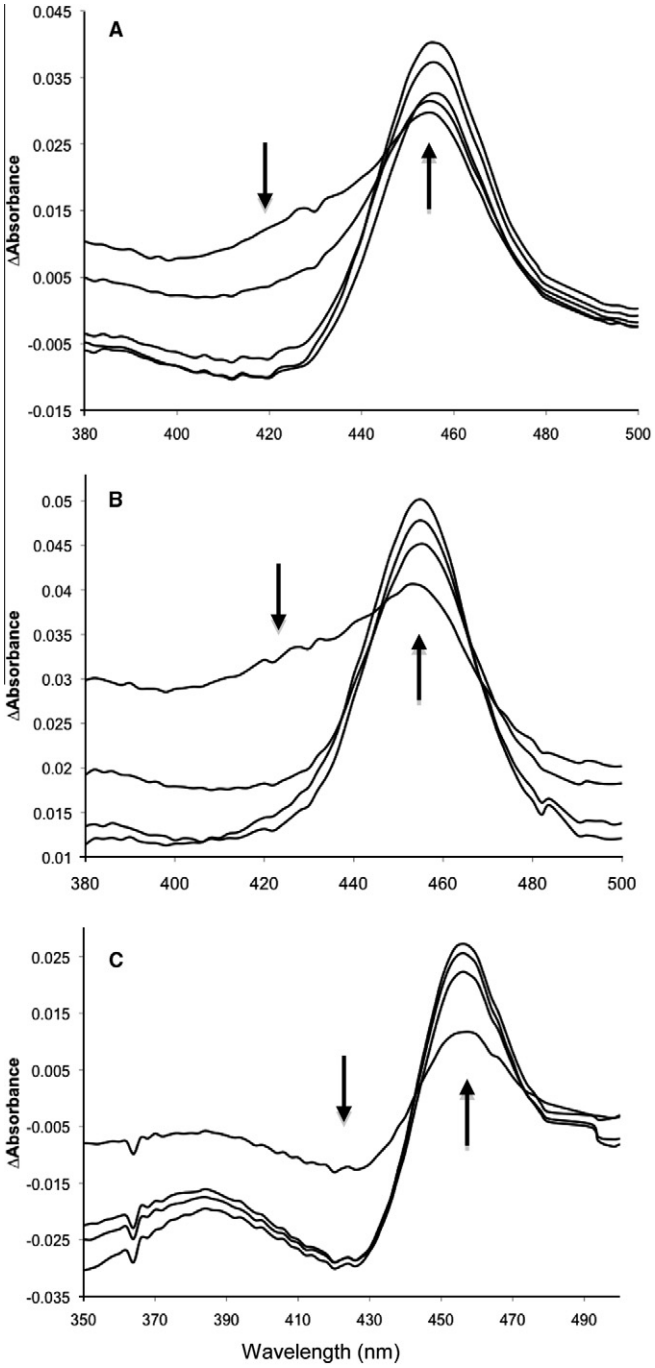

Figure 2. Difference spectra of the binding of (A) oleic acid, (B) linoleic acid, and (C) arachidonic acid to MPO. Each curve is the result of subtracting the spectrum of MPO–lipid from MPO only at increasing lipid concentrations during the titration of MPO with lipid (final concentration of lipid 10–100 μM). The difference spectra showed an increasingly larger difference between A_{max} and A_{min} after each consecutive addition of lipid to MPO.

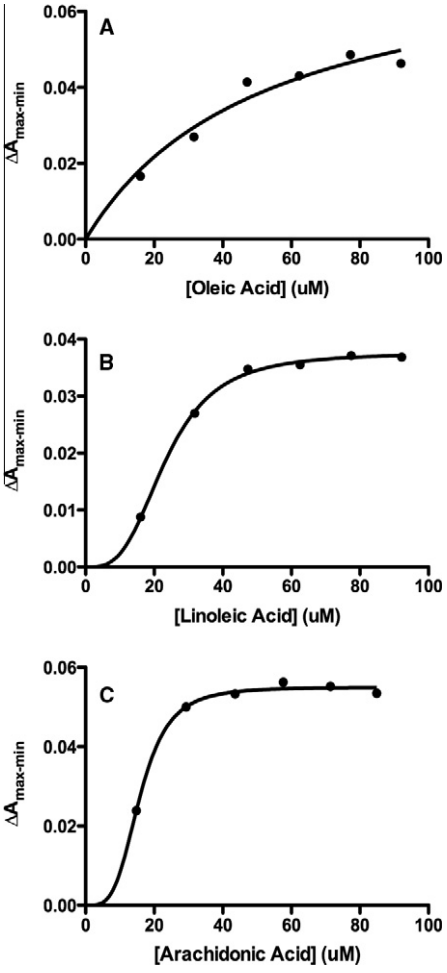

Figure 3. Binding curves of oleic acid (A), linoleic acid (B), and arachidonic acid (C) with MPO. The change in absorbance ($\Delta A_{max-min}$) was determined from difference spectra of MPO–lipid versus MPO only; graphs are an average of a minimum of three independent trials.

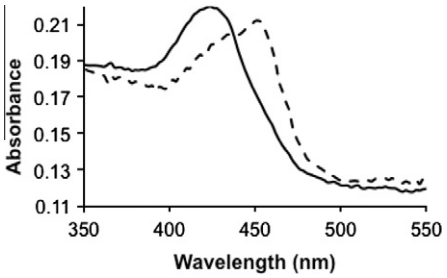

Figure 4. Spectrum of MPO (solid line) and compound **II** (dashed line), formed after addition of H_2O_2 in fivefold molar excess.

Other heme containing proteins, including sperm whale myoglobin and *Vitrioscilla* hemoglobin, have been shown to bind lipids in the distal heme pocket.[16–18] The absorption shift for these heme proteins upon binding of substrate has been hypothesized as the result of the interaction of *p* orbitals of the *cis* double bond of the fatty acid with a *d* orbital of heme's ferric iron. The results in this study, therefore, indicate close proximity of the alkene to the oxidizing moiety, the iron atom, of MPO. The difference in binding affinities of the three individual lipids (oleic, linoleic, and arachidonic acid) can be attributed to the number of *cis* bonds in the lipid, which in turn causes the lipid to be more compact in size as the number of double bonds increases. While oleic has one double bond, producing a simple kink in the carbon chain, both linoleic and arachidonic acids have a more rigid and conformationally constrained structure due to the steric constraints of their multiple double bonds. The tighter binding of linoleic and arachidonic acid to MPO, therefore, may be attributed to a binding site that can accommodate the more compact lipids. Additionally, the presence of multiple double bonds in both linoleic and arachidonic acid may contribute to a higher likelihood of binding, simply because there

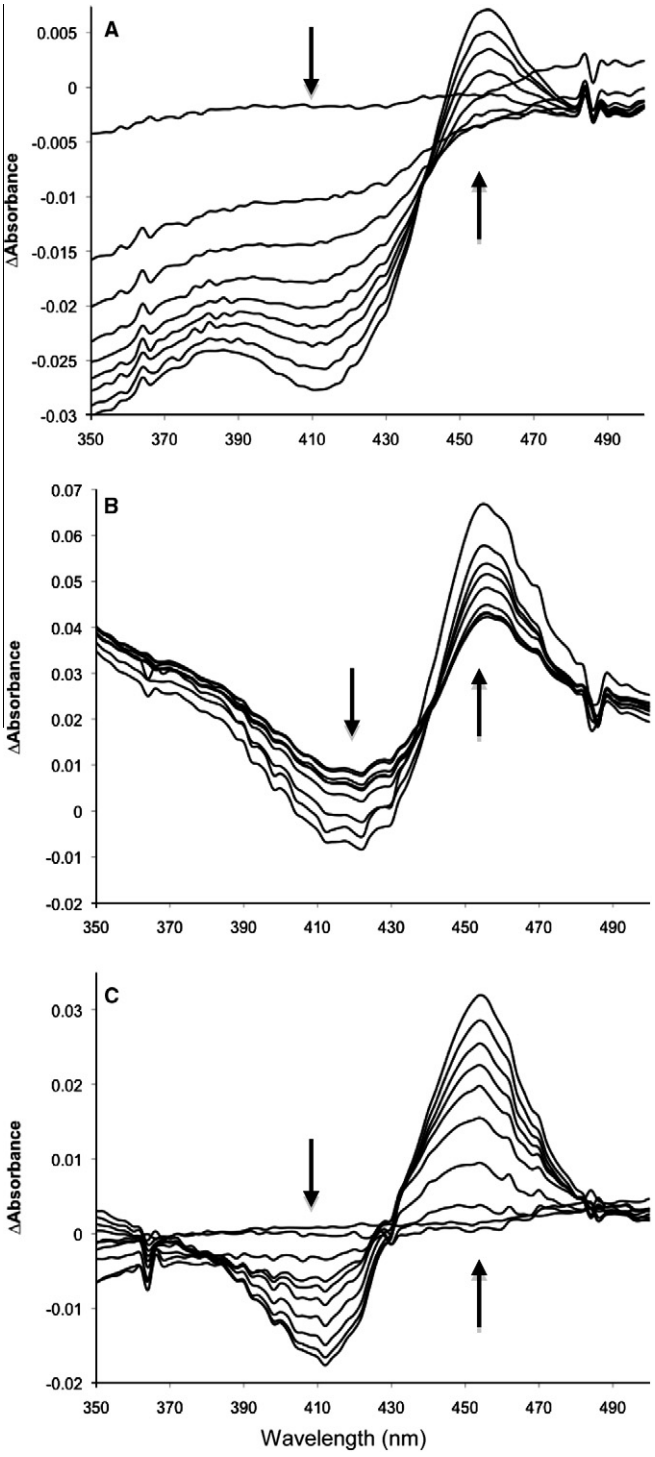

Figure 5. Difference spectra of the oxidation of (A) oleic acid, (B) linoleic acid, and (C) arachidonic acid with MPO (2 μM) in the presence of hydrogen peroxide (10 μM). Each curve is the result of subtracting the spectrum of the reaction between compound **II** and lipid from compound **II** only in 20 s intervals over a period of 3 min. For each lipid, one experiment using a final lipid concentration of either 0.5 mM or 1.0 mM is shown in the figure.

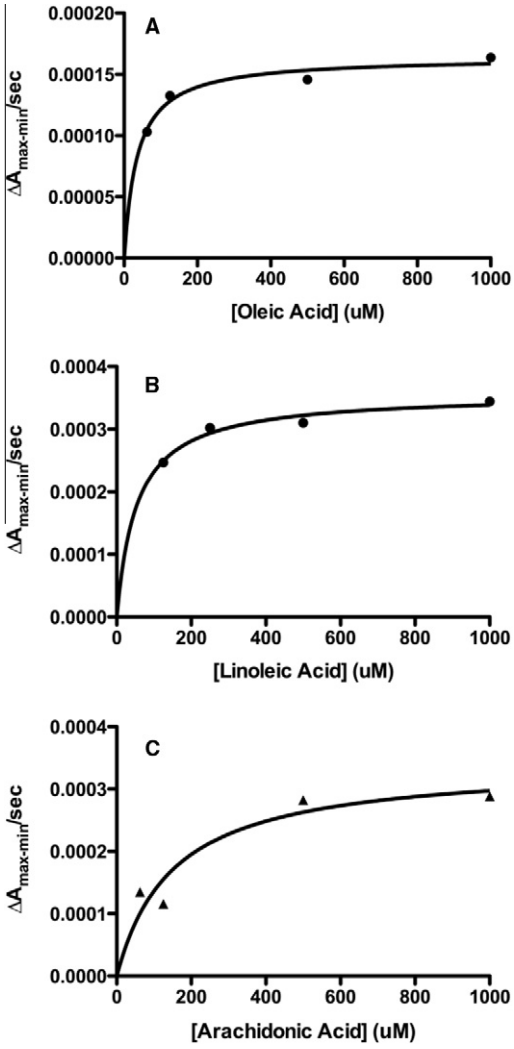

Figure 6. Reaction of compound **II** of myeloperoxidase (MPO) with (A) oleic acid, (B) linoleic acid, and (C) arachidonic acid. The change in absorbance per second ($\Delta A_{max-min}/s$) was determined from difference spectra of MPO–lipid versus MPO only measured over 3 min in 2 s intervals. Initial velocities were determined from the linear portion of the rate graphs, typically the first 1–2 min, for each lipid concentration. A minimum of three independent trials at each lipid concentration was used to determine $\Delta A_{max-min}/s$.

are more potential sites on the lipid that can bind to MPO. This observation correlates with the notion that the alkene functionality plays a large role in binding of fatty acid to MPO, as suggested by other heme protein binding studies. Interestingly, both linoleic and arachidonic acid's sigmoidal binding curves suggest that more than one molecule of each of these lipids may bind to MPO in a cooperative binding fashion, whereas oleic's binding data indicates a simple one to one ratio of lipid to enzyme. Because myeloperoxidase is a dimer containing two heme groups, the possibility of binding more than one lipid per myeloperoxidase molecule is conceivable; however, the Michaelis–Menton kinetics observed for these lipids would argue for a one to one lipid to enzyme oxidation relationship.

A relationship between degree of unsaturation on the fatty acid chain and rate of oxidation by MPO as measured by k_{cat} was demonstrated, with faster rates of oxidation correlating to linoleic and arachidonic acids, which both have bis-allylic positions, as opposed to oleic acid, which does not. Reduction of compound II to native ferric MPO does not occur without lipid; oxidation of lipid does not occur without both MPO and hydrogen peroxide present. Spectroscopic measurements of MPO and hydrogen peroxide together at the same concentrations used for kinetic studies were obtained without fatty acid present. Compound **II** appears to decay in a time dependent manner; however, the concentration of native ferric MPO does not increase substantially (Fig. 7). While previous research reports suggest that superoxide, generated from the

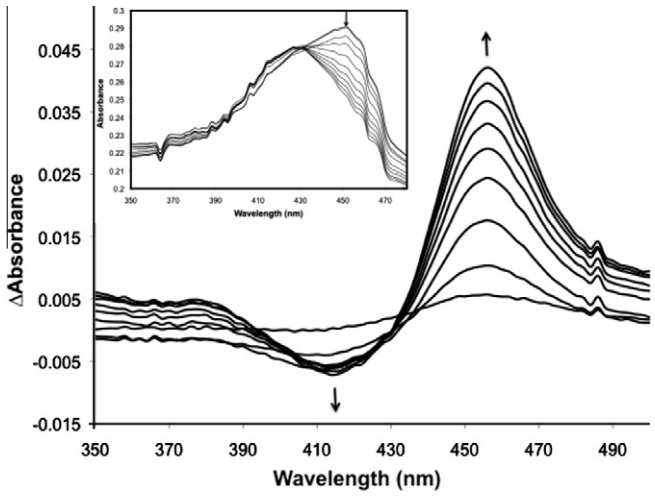

Figure 7. Difference spectra of the decay of compound **II**. Compound **II** was generated by adding excess H_2O_2 (10 μM) to MPO (2 μM). Each difference spectra was generated by subtracting the spectrum in 20 s intervals from the initial compound **II** spectrum. Inset shows the actual spectral changes of the Soret band. Compound **II** (456 nm) decays without concomitant increase of ferric MPO (426 nm).

oxidation of hydrogen peroxide during the reduction of compound I to **II**, may reduce compound II to the ferric MPO without the presence of an additional substrate, no evidence of this side reaction was observed.[23] When lipid is present, the absorbance corresponding to compound II decreases while simultaneously the absorbance corresponding to ferric MPO increases, as seen in the difference spectra (Fig. 5), suggesting the direct one-electron oxidation of lipid by compound **II**.

Reaction of fatty acid and hydrogen peroxide without MPO present, again using the same concentrations as in the kinetic spectroscopic studies, showed no evidence of oxidation products when examined by silica gel thin layer chromatography. Analysis by thin layer chromatography (development using a mixture of 80:20:1 hexane/diethyl ether/acetic acid) after 30 min reaction time between the individual lipids and hydrogen peroxide and subsequent extraction by hexane or dichloromethane, revealed only unreacted lipid (data not shown). Oxidized lipid would travel more slowly on a silica gel plate because of the polar nature of the oxygen functionalities introduced into the lipid chain during oxidation; oxidized products were observed by thin layer chromatography when MPO was included in the reaction mixture with hydrogen peroxide and lipid.

The ease of oxidation of these fatty acids with MPO appears to correlate with the proximity of the hydrogens on the allylic carbon to the iron atom of the heme, as suggested by the binding studies. These hydrogens are susceptible toward free radical oxidation because of the resulting resonance stability of an allylic radical once a hydrogen radical has been removed (Fig. 8). Therefore, unsaturated fatty acids are more likely to be oxidized than saturated fatty acids. The data obtained from this experiment show that the two lipids containing bis-allylic positions, linoleic and arachidonic acids, in fact, are oxidized at twice the rate of oleic acid, which corresponds to both the presence of a reactive hydrogen atom and increased binding affinity of these two lipids over oleic acid. The specificity constant k_{cat}/K_M, suggests linoleic acid is most efficiently oxidized in the presence of MPO, followed by oleic and then arachidonic acid; however, this only gives a measurement for the second of the two oxidative steps in the MPO enzyme cycle. To fully explore the overall oxidation efficiency of each lipid with MPO in the complete catalytic cycle, further kinetic experiments will need to be carried out.

Free fatty acids and their oxidized products do not bind competitively to CD36 receptors. Instead, a structural motif resulting from oxidation of a choline glycerophospholipid, such as 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphocholine, appears to be necessary for recognition by the receptors on macrophages.[5] This motif, consisting of a *sn*-2-esterified-γ-hydroxy(or oxo)-α,β-unsaturated carbonyl containing chain, is the result of at least one pathway of MPO oxidation. The entire oxidized choline glycerophospholipid is necessary for macrophage recognition and subsequent engulfing to form foam cells; however, the unsaturated fatty acid side chain at carbon 2 of the glycerophospholipid appears to be the region which actually binds to MPO to initiate oxidation. Oleic or palmitic acid are typically found at carbon 1 of the glycerol backbone; no evidence of either fatty acid chain oxidizing while at carbon 1 has been reported. Arachidonic and linoleic acids are predominantly located at carbon 2, and have been shown in this Letter to bind preferentially over the monounsaturated oleic acid. Not surprisingly, then, that the lipid at carbon 2 on the glycerol backbone provides the marker for macrophage recognition as this chain is most likely to be bind and subsequently oxidize.

The mechanism of oxidation of lipids in the presence of MPO, in particular in vivo, has been debated. Free radical oxidation is assumed, although whether this occurs through tyrosyl radicals generated on MPO, peroxy nitrate compounds derived from nitric oxide, or via hypohalous acids, all of which have been implicated in initiating oxidation of lipids, has been discussed. Presumably, multiple pathways of both one- and two-electron oxidative mechanisms exist in which lipids are ultimately oxidized in vivo. The results of oxidation with only MPO, hydrogen peroxide and fatty acid, in the absence of other oxidizable substrates, however, demonstrate that not only do the lipids bind directly with high affinity to MPO, but are oxidized through a one-electron free radical pathway most likely at the allylic position. A possible mechanism for oxidation of fatty acid lipids by heme proteins has been suggested in which the ferryl oxygen of compound II abstracts an allylic proton and electron from the bound fatty acid, resulting in a one-electron reduction of Fe(IV) to Fe(III) and formation of an iron coordinated hydroxyl group (Fe(III)–OH).[16] Rapid rearrangement of the remaining allylic radical on the fatty acid yields a conjugated diene radical for both arachidonic and linoleic acid. Because of the proximity of the hydroxyl group on Fe(III) to the bound fatty acid, transfer of a hydroxyl radical to the conjugated radical would result in one of the major observed products of fatty acid oxidation, and the core of the structural motif deemed necessary for macrophage uptake of oxLDL: a γ-hydroxyl group to an α,β-unsaturated system (Fig. 8). This transfer, however, would leave a radical

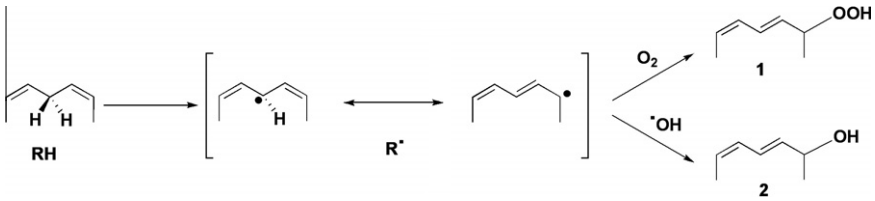

Figure 8. Formation of oxidized lipid products, hydroperoxide (**1**) and hydroxy (**2**) lipids, through conjugated diene radical intermediate.

associated with the heme and not return compound **II** to its neutral native ferric iron oxidation state. More likely, therefore, the allylic radical is directly attacked by oxygen in the binding pocket to form a hydroperoxide, another product observed in the oxidation of lipids.[24] In either oxidative mechanistic scenario, the lipid likely remains bound to the enzyme during oxygen attack, which would prevent premature release of a free radical. This binding affinity helps explain the lack of benefit of the antioxidants, vitamin E and β-carotene, in clinical trials with regard to cardiovascular outcomes.[25] The fatty acid chains of LDL can be oxidized entirely within the confines of the binding site of MPO, removed from the reductive effect of an antioxidant.

Previous research has shown that (1) MPO binds to LDL and (2) oxidized fatty acid chains are a key recognition feature on the intact glycerophospholipid for uptake of LDL by macrophage receptors. This Letter has shown the MPO will bind the fatty acids found in glycerophospholipids of LDL and has suggested that the lipid is oxidized via a one-electron mechanism. Because the fatty acid chains on the glycerophospholipids are known to be oxidized before uptake by receptors, the results of this Letter suggest that MPO binds fatty acid chains on intact glycerophospholipid molecules in the LDL monolayer to oxidize the fatty acid chains before uptake by macrophages. Investigations into the glycerophospholipid's propensity for binding to and oxidation by MPO are underway.

The relationship between alkene content in lipids and oxidized LDL has been known for over 15 years. Populations fed diets rich in oleic acid as opposed to linoleic acid generate less oxLDL and show less degradation by macrophages.[26,27] The results shown in the current in vitro study concur with what has been observed in vivo. One simple method for reducing uptake of oxLDL by macrophages and therefore the initiation of atherosclerosis may be to change the lipid composition in LDL by altering diet. Alternatively, MPO inhibitors can be designed that prevent or lessen the binding of polyunsaturated molecules. Studies are underway to clarify the mechanism of oxidation of lipids directly by MPO and examine potential inhibitors for MPO–lipid oxidation, which will allow for focused therapeutic strategies to lessen the formation of oxidized LDL.

Acknowledgments

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References and notes

1. Ehara, S.; Ueda, M.; Naruko, T.; Haze, K.; Itoh, A.; Otsuka, M.; Komatsu, R.; Matsuo, T.; Itabe, H.; Takano, T.; Tsukamoto, Y.; Yoshiyama, M.; Takeuchi, K.; Yoshikawa, J.; Becker, A. E. *Circulation* **2001**, *103*, 1955.
2. Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. N. *Engl. J. Med.* **1989**, *320*, 915.
3. Steinberg, D. *J. Lipid Res.* **2009**, *50*, S376.
4. Podrez, E. A.; Febbraio, M.; Sheibani, N.; Schmitt, D.; Silverstein, R. L.; Hajjar, D. P.; Cohen, P. A.; Frazier, W. A.; Hoff, H. F.; Hazen, S. L. *J. Clin. Invest.* **2000**, *105*, 1095.
5. Podrez, E. A.; Poliakov, E.; Shen, Z.; Zhang, R.; Deng, Y.; Sun, M.; Finton, P. J.; Shan, L.; Gugiu, B.; Fox, P. L.; Hoff, H. F.; Salomon, R. G.; Hazen, S. L. *J. Biol. Chem.* **2002**, *277*, 38503.
6. Chisolm, G. M., III; Hazen, S. L. *J. Biol. Chem.* **1999**, *274*, 25959.
7. Stocker, R.; Keaney, J. F., Jr. *Physiol. Rev.* **2004**, *84*, 1381.
8. Zhang, R.; Brennan, M.-L.; Shen, Z.; MacPherson, J. C.; Schmitt, D.; Molenda, C. E.; Hazen, S. L. *J. Biol. Chem.* **2002**, *277*, 46116.
9. Zhang, R.; Brennan, M. L.; Fu, X.; Aviles, R. J.; Pearce, G. L.; Penn, M. S.; Topol, E. J.; Sprecher, D. L.; Hazen, S. L. *J. Am. Med. Assoc.* **2001**, *286*, 2154.
10. Nicholls, S. J.; Hazen, S. L. *J. Lipid Res.* **2009**, *50*, S346.
11. Jayaraman, S.; Gantz, D. L.; Gursky, O. *Biochemistry* **2007**, *46*, 5790.
12. Terpstra, V.; Bird, D. A.; Steinberg, D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1806.
13. Malle, E.; Marsche, G.; Arnhold, J.; Davies, M. J. *Biochim. Biophys. Acta* **2006**, *1761*, 392.
14. Exner, M.; Hermann, M.; Hofbauer, R.; Hartmann, B.; Kapiotis, S.; Gmeiner, G. *Free Radical Biol. Med.* **2004**, *37*, 146.
15. Carr, A. C.; Myzak, M. C.; Stocker, R.; McCall, M. R.; Frei, B. *FEBS Lett.* **2001**, *487*, 176.
16. Rao, S. I.; Wilks, A.; Hamberg, M.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1994**, *269*, 7210.
17. Bonamore, A.; Farina, A.; Gattoni, M.; Schinina, M. E.; Bellelli, A.; Boffi, A. *Biochemistry* **2003**, *42*, 5792.
18. Rinaldi, A. C.; Bonamore, A.; Macone, A.; Boffi, A.; Bozzi, A.; Di Giulio, A. *Biochemistry* **2006**, *45*, 4069.
19. Jantschko, W.; Furtmuller, P. G.; Allegra, M.; Livrea, M. A.; Jakopitsch, C.; Regelsberger, G.; Obinger, C. *Arch. Biochem. Biophys.* **2002**, *398*, 12.
20. Ghibaudi, E.; Laurenti, E. *Eur. J. Biochem.* **2003**, *270*, 4403.
21. Furtmuller, P. G.; Zederbauer, M.; Jantschko, W.; Helm, J.; Bogner, M.; Jakopitsch, C.; Obinger, C. *Arch. Biochem. Biophys.* **2006**, *445*, 199.
22. Ghibaudi, E.; Laurenti, E.; Pacchiardo, C.; Suriano, G.; Moguilevsky, N.; Ferrari, R. P. *J. Inorg. Biochem.* **2003**, *94*, 146.
23. Winterbourn, C. C.; Hampton, M. B.; Livesey, J. H.; Kettle, A. J. *J. Biol. Chem.* **2006**, *281*, 39860.
24. Folcik, V. A.; Cathcart, M. K. *J. Lipid Res.* **1994**, *35*, 1570.
25. Kris-Etherton, P. M.; Lichtenstein, A. H.; Howard, B. V.; Steinberg, D.; Witztum, J. L. *Circulation* **2004**, *110*, 637.
26. Reaven, P.; Parthasarathy, S.; Grasse, B. J.; Miller, E.; Almazan, F.; Mattson, F. H.; Khoo, J. C.; Steinberg, D.; Witztum, J. L. *Am. J. Clin. Nutr.* **1991**, *54*, 701.
27. Reaven, P.; Parthasarathy, S.; Grasse, B. J.; Miller, E.; Steinberg, D.; Witztum, J. L. *J. Clin. Invest.* **1993**, *91*, 668.