Selective oxidation in vitro by myeloperoxidase of the N-terminal amine in apolipoprotein B-100

Chao-yuh Yang,[1,*,†] Jin Wang,[*,†] Andrew N. Krutchinsky,[§] Brian T. Chait,[§] Joel D. Morrisett,[*,†] and Charles V. Smith[**]

Department of Biochemistry* and Department of Medicine,† Baylor College of Medicine, Houston, TX; Rockefeller University,§ New York, NY; and Children's Research Institute,** Ohio State University, Columbus, OH

Abstract **In contrast to the multiple low abundance 2,4-dinitrophenylhydrazine-reactive tryptic peptides formed by oxidation of LDL with reagent HOCl in vitro, myeloperoxidase-catalyzed oxidation produces a dominant product in considerably greater yield and selectivity. This modified peptide had a single amino-terminal sequence corresponding to amino acids 53–66 of apolipoprotein B-100 (apoB-100), but its mass spectra indicated a significantly higher mass than could be reconciled with simple modifications of this peptide. Subsequent studies indicate that this product appears to result from N-chlorination of the N-terminal amino group of apoB-100 and dehydrohalogenation to the corresponding imine, which may form the hydrazone derivative directly, or after hydrolysis to the ketone. The methionine residue is oxidized to the corresponding sulfoxide, and the primary sequence peptide (residues 1–14 of apoB-100) is linked by the intramolecular disulfide bond between C-12 and C-61 to the peptide composed of residues 53–66, as we have observed previously (**Yang, C-Y., T. W. Kim, S. A. Weng, B. Lee, M. Yang, and A. M. Gotto, Jr. **1990. *Proc. Natl. Acad. Sci. USA.*** 87: **5523–5527) in unmodified LDL. The selective oxidation by myeloperoxidase of the N-terminal amine suggests strong steric effects in the approach of substrate to the enzyme catalytic site, an effect that may apply to other macromolecules and to cell surface molecules.**—Yang, C-y., J. Wang, A. N. Krutchinsky, B. T. Chait, J. D. Morrisett, and C. V. Smith. **Selective oxidation in vitro by myeloperoxidase of the N-terminal amine in apolipoprotein B-100. *J. Lipid Res.*** **2001.** 42: **1891–1896.**

Supplementary key words 2,4-dinitrophenylhydrazine • low density lipoprotein oxidation • protein carbonyl • protein oxidation

A considerable body of evidence indicates that oxidative modifications of LDL are involved in atherosclerosis (1–4), and myeloperoxidase (MPO)-catalyzed reactions are among the principal mechanisms considered for this oxidation of LDL (5). Oxidation of LDL has been studied extensively, but the great majority of these studies have focused on products resulting from oxidation of the lipid components (6–8).

Characterization of products of oxidation of the protein component, apolipoprotein B-100 (apoB-100), has received increasing attention. However, most of these studies have used total hydrolysis procedures that do not distinguish residues by position in the primary structure, as will be required for product identification to address important questions regarding the effects of the intact LDL particle on its interaction with specific oxidants. We have been working to characterize the specific products of oxidative modification of the LDL apolipoprotein, apoB-100, in order to distinguish among the mechanisms whereby LDL is oxidized in vivo (9–11). In our initial studies of the oxidation of LDL with reagent HOCl, followed by treatment with 2,4-dinitrophenylhydrazine (DNPH), intending to convert the presumed aldehydes and ketones formed by HOCl-driven oxidation of the protein, we instead found that most of the 14 products we characterized indicated modifications of cysteine or tryptophan residues (10). Subsequent studies indicated that the DNPH-reactive products of oxidation of the cysteine residues were best interpreted as the respective sulfinic acids (9). Additional studies of Cu^{2+}-catalyzed oxidation of LDL revealed tryptic peptide products of oxidation of the apolipoprotein that absorbed at 365 nm, but in a manner not dependent on derivatization with DNPH (11). These products were identified as the kynurenines corresponding to oxidation of tryptophan residues in apoB-100.

Although the oxidation of biological molecules by MPO is commonly attributed to generation of HOCl (5, 12–17), we observed that MPO-catalyzed oxidation of LDL in vitro produced a much different pattern of DNPH-reactive products than was observed from reaction of LDL with reagent HOCl (9, 10). Oxidation of LDL with HOCl produced a diverse array of DNPH-reactive peptides, with little indication of selectivity. We isolated and characterized 14 of

Abbreviations: apo, apolipoprotein; DNPH, 2,4-dinitrophenylhydrazine; ESI, electrospray ionization; MALDI, matrix-assisted laser desorption/ionization; MPO, myeloperoxidase.

[1] To whom correspondence should be addressed at the Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.
e-mail: cyang@bcm.tmc.edu

these modified peptides, the majority of which were derived from oxidation of cysteine residues located in peptides that are released by limited tryptic digestion of intact LDL particles, suggesting that these sequences are located on the surface of the LDL particle (10).

In contrast, MPO-mediated oxidation of LDL in vitro, derivatization with DNPH, and tryptic digestion produced a dominant peptide detected by absorbance at 365 nm (9). Formation of this peptide required MPO, H_2O_2, and Cl^-. Our previous efforts to characterize this oxidized peptide were only partly successful, with gas-phase sequencing indicating a single N-terminal sequence, VELEVPQL(*C)SFILK . . . , corresponding to amino acid residues 53 through 66 in apoB-100. Mass spectral studies were consistent with these studies, but did not enable us to characterize fully the structure of the modified peptide. Although our previous studies showed clearly that MPO-catalyzed oxidation of LDL is not attributable simply to free solution interactions of HOCl with LDL (10), the larger goal of our studies of the oxidation of the apolipoprotein as a means to identify the specific mechanisms responsible for oxidative modifications of LDL in vivo requires characterization of products on equally specific bases. The studies described in the present report have enabled us to identify the peptide that we had previously characterized only partially. The structure of the product peptide suggests a plausible explanation for the mechanisms of interactions of MPO with LDL. In addition to important implications for development of specific biomarkers of inflammatory modification of LDL in vivo, the present data also suggest potentially important mechanistic implications for the interactions of MPO with cell surface molecules on invading pathogens and other biological substrates.

MATERIALS AND METHODS

Materials

MPO (human polymorphonuclear leukocytes) was purchased from CalBiochem (San Diego, CA), hydrogen peroxide (30%) was from Fisher Scientific (Pittsburgh, PA), DNPH was from Serva (Heidelberg, Germany) or from Sigma (St. Louis, MO), and *m*-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-treated trypsin was from Worthington Biochemicals (Freehold, NJ). All other reagents were purchased from Sigma.

LDL preparation

LDL was isolated from plasma of fasting healthy donors by sequential ultracentrifugation in KBr solution at densities between 1.030 and 1.063 g/ml, as we have described previously (10). Briefly, aprotinin (0.056 unit/ml plasma), sodium azide (0.06%, w/v), and EDTA (0.06%, w/v) were added to plasma immediately after collection. For additional purification, LDL was recentrifuged to d = 1.09 g/ml. Purified LDL was dialyzed against 50 mM phosphate buffer containing 150 mM NaCl, pH 7.4 (50 mM PBS).

Oxidation of LDL by MPO

Oxidation of LDL by MPO was performed according to the method described by Hazell and Stocker (17) with some modification. LDL in 50 mM PBS at a final concentration of 0.8 μM apolipoprotein was preincubated at 37°C for 10 min before addition of MPO at a final concentration of 1.2 μg/ml. Oxidation was initiated by addition of H_2O_2 at 1.5-min intervals, with brief mixing after each addition, to a final concentration of 10 μM. Under these conditions, H_2O_2 was consumed by MPO within 1 min. Samples were protected from light between additions of H_2O_2. The total incubation time was 15 min and the sample was placed in ice for further processing. Replacement of the 50 mM PBS with a 50 mM phosphate buffer, without Cl^-, also was used in control studies of the oxidation of LDL by MPO-H_2O_2.

In additional experiments, 1.6 nmol of LDL and 0.02 nmol of MPO in 2 ml of 50 mM PBS were incubated at 37°C for 10 min. Oxidation of LDL was effected by addition of 10 equal aliquots, 1.5 min apart, each of 2 nmol of H_2O_2 in 50 μl of PBS. Similar oxidations were conducted at 37°C in the presence or absence of MPO by addition of 16, 40, 160, 400, 1,000, or 4,000 nmol of HOCl, either added at 1.5 min intervals in 10 separate aliquots or as a single bolus dose, corresponding to an HOCl:apoB-100 molar ratio of 10, 25, 100, 250, 625, and 2,500, respectively. Fifteen minutes after initiation of the respective oxidations, the samples were reacted with DNPH, the protein was precipitated, delipidated, and digested with trypsin, and the tryptic peptides were analyzed by HPLC, as we have described previously (9).

Reaction of LDL with DNPH

The reaction of LDL with DNPH was performed as described (9). An equal volume of DNPH (5 mM in 2 N HCl) was added to the LDL. After incubation for 30 min at 37°C, 1 volume of 20% TCA was added. The precipitate was collected by centrifugation and washed with ethanol–ethyl acetate 1:1 (v/v) three times and dried with nitrogen.

Protein primary structure analysis

Delipidation and tryptic cleavage were conducted as described previously (9). Proteins were digested with TPCK-treated trypsin (enzyme:substrate ratio = 1:50) in 0.1 M ammonium bicarbonate, pH 8.0, at room temperature for up to 24 h. Peptides were separated by reversed-phase HPLC for sequencing. A Vydac (Hesperia, CA) C_{18} column (250 × 4.6 mm) and trifluoroacetic acid-acetonitrile gradient elution was used for primary peptide separations. The major peaks detected by absorbance at 365 nm, which is characteristic of the dinitrophenyl group, were collected manually and purified by sequential chromatography with phosphate buffer and ammonium acetate-acetonitrile gradient mobile phases (9). The sequences of the peptides thus obtained were determined with a gas-phase automated sequencer (Applied Biosystems, Foster City, CA) with an online 120A PTH analyzer.

Mass spectrometry

New interface for the QqTOF mass spectrometer. The Sciex (Concord, Canada) prototype QqTOF instrument (Centaur) was modified by the addition of an ion source interface that enables us to operate in either matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) mode, with the option of changing quickly between these two modes (18).

Electrospray mode. The core of the modification is an insert between the first quadrupole (q0) and the skimmer, where we have added a small quadrupole (q00). This additional quadrupole acts as the first collisional ion guide (19). When operating in the ESI mode, the MALDI probe is removed and ions that are produced in the standard nanoelectrospray ion source (Protana, Odense, Denmark) are introduced into the instrument through the standard orifice plate and skimmer into q00, and then through a small gap (0.8 cm) into the original q0 quadrupole ion guide (20).

MALDI mode. The MALDI probe inserts into the gap in the electrode that separates the q00 and q0 quadrupoles (20). The cylindrical tip of the probe (diameter, 0.5 cm) accommodates ~40 samples on its surface. The beam of a nitrogen laser operating at a repetition rate of 20 Hz is introduced through a quartz window at an angle of ~30° to the surface of the sample. The laser beam is focused by a lens (f = 25 cm) to an ~0.2- to 0.3-mm-diameter spot. The power density of the laser radiation in the spot was varied between 10^7 and 10^8 W/cm^2 through fine control of the focal diameter of the laser beam. No discernible increase in the level of metastable decay was observed under these relatively high fluence laser conditions. The position of each sample was manually adjusted at the entrance of q0 by translating and rotating the probe. Both the sample and laser spots were monitored by a video camera. Desorbed ions were introduced directly into q0. No voltage changes were required when changing from ESI to MALDI mode (20).

RESULTS

MALDI spectra of the major DNPH-reactive peptide produced by MPO-catalyzed oxidation of LDL, derivatization with DNPH, and digestion with trypsin [labeled 1C-12-1-1 in ref. (9)] suggested a mass of about 3,400 Da (data not shown). The MALDI spectra were not readily interpretable in terms of a structure for an oxidative modification of the peptide identified by N-terminal sequence analyses. Even in light of the structure indicated by our subsequent and more definitive ESI-tandem mass spectrometry (MS/MS) analyses, assignment or rationalization of ions from the MALDI spectra has not been possible. In contrast, ESI spectra of the peptide gave distinct ions at *m/z* 1,143.886 (3+) and 858.164 (4+), which indicated a mass for the peptide of 3,428.658 (**Fig. 1**).

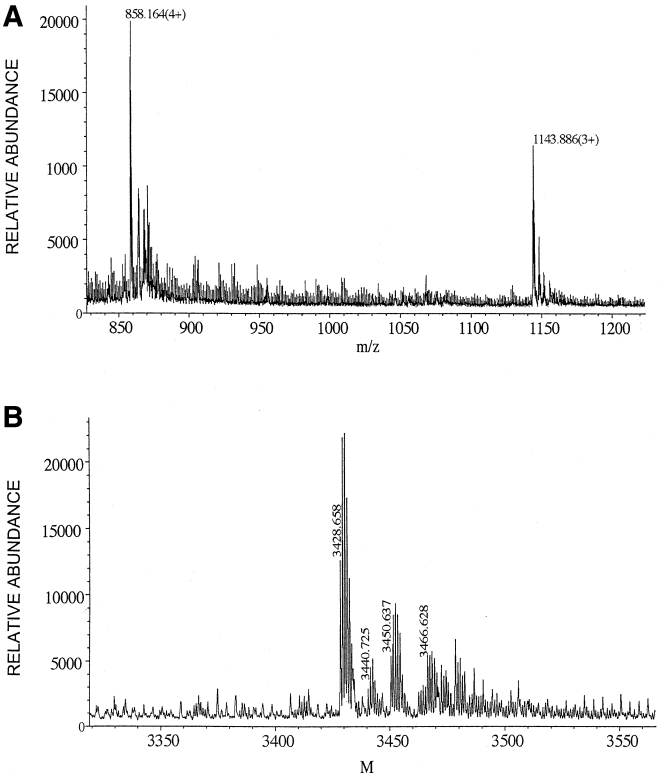

Fig. 1. ESI mass spectra of the major modified peptide from MPO-catalyzed oxidation of LDL in vitro. A: ESI-MS/MS of the peptide gave prominent 4+ ions at *m/z* = 858.164 and +3 ions at 1,143.886. B: These ions indicate a monoisotopic mass of 3,428.658.

The lower *m/z* region of the MS/MS fragmentation spectra of the *m/z* = 1,143.886 (3+) ion revealed a sequence of b series ions that are consistent with residues 53–59 of apoB-100 (**Fig. 2A**). The higher mass region of the MS/MS spectrum of the 1,143.886 (3+) ion showed another peptide fragment ion sequence consistent with doubly charged y′′ fragment ions from amino acids 5–11 (LENVSLV) of apoB-100 (Fig. 2B). In addition, the VEL fragment sequence observed in this spectrum is consistent with doubly charged y′′ ions from residue 55 to 57, with the VE residues (53 and 54, respectively) implied by the mass difference indicated by the ion at *m/z* = 1,601.273 (z = 2+) and the mass of 3,428.658 indicated for the parent compound (Fig. 1). The calculated mass of the two tryptic peptides indicated by these sequences, amino acids 1–14 (EEEMLENVSLVCPK) and 53–66 (VELEVPQLCSFILK) of apoB-100, joined by a disulfide bond between the respective C residues, is 3,233.6, or 195 Da lower than is observed with the modified peptide. We have described

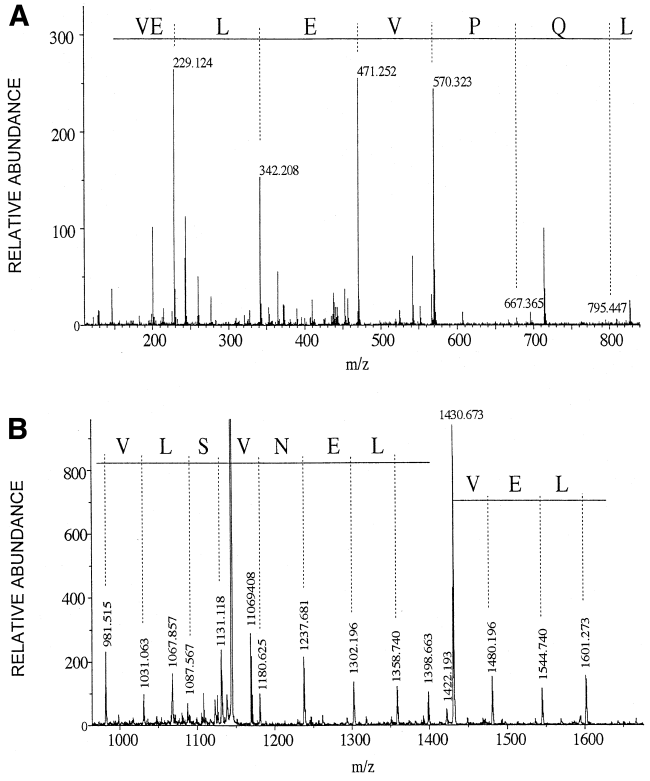

Fig. 2. MS/MS spectra of *m/z* = 1,143.886 (3+). A: Low *m/z* region MS/MS spectrum of the (+3) ion at *m/z* = 1,143.886 gave b series fragment ions consistent with the N-terminal sequence data observed previously (10) by gas-phase sequence analysis. B: High *m/z* region MS/MS spectrum of doubly charged fragment ions indicated, in addition to the partial sequence of y fragment ions of VELE . . . , a series of y fragment ions characteristic of the peptide sequence found in the N-terminal tryptic peptide of apoB-100.

previously the linkage in native LDL of these two peptide sequences by a disulfide bond between their respective C residues (21).

MS/MS spectra of the ion at m/z = 858.164 (4+) indicated oxidation of the M4 residue in the apolipoprotein (**Fig. 3A**), thus accounting for a mass increase of 16 Da. The absence of a significant ion at m/z 1041 in this sequence, as would be expected from the continuation of y" fragmentation through the E at the second residue in apoB-100, suggests either that E2 is modified or that the normal sequence of y" fragmentation is compromised by chemical modification of E1 (the N-terminal E). The ion at m/z = 1,067 Da has not been assigned at this time, but may not be a member of this series of fragments. The low m/z region of the MS/MS fragmentation spectrum of 858.164 (4+) shows a series of singly charged b series fragments of residues 53–57 (Fig. 3B) and a second series corresponding to residues 3–5, but shifted by 179 Da to masses higher than calculated for the unmodified sequence. The ion at m/z = 438.097 is consistent with the net increase in mass of the parent ion being localized to the two N-terminal E residues and the corresponding b_2 ion suggests that E2 is intact. The ion at m/z = 309 Da that would be expected from fragmentation of E2 from the b_2 ion at m/z = 438 is not observed with appreciable intensity.

The selectivity of the oxidation of LDL with reagent HOCl was examined in greater detail, including oxidation at 37°C and in the presence of MPO, using a range of doses of HOCl from 10 to 625 mol of HOCl per mol of LDL (**Fig. 4**). The product profile formed with 12.5 mol of H_2O_2 per mol of LDL in the MPO-catalyzed reaction is included for comparison. The absence of any detectable (at a signal:noise ratio of 2:1) amount of the major modified peptide eluting at 35 min with 10 equivalents of HOCl contrasts sharply with the signal:noise ratio of approximately 50:1 shown in the MPO plus H_2O_2 oxidation. Additional experiments showed no differences between the presence and absence of MPO in LDL oxidations by HOCl (data not shown).

The data are most consistent with the interpretation that the major DNPH-reactive peptide [1C-12-1-1 from ref. (9)] is the DNPH-derived hydrazone formed by oxidation of the N-terminal amino group of apoB-100. This interpretation, in turn, is most straightforwardly rationalized by initial formation of the N-terminal chloramine of apoB-100 by MPO-catalyzed oxidation, followed by dehydrohalogenation to the imine, which may react directly with DNPH to form the hydrazone or may hydrolyze to the

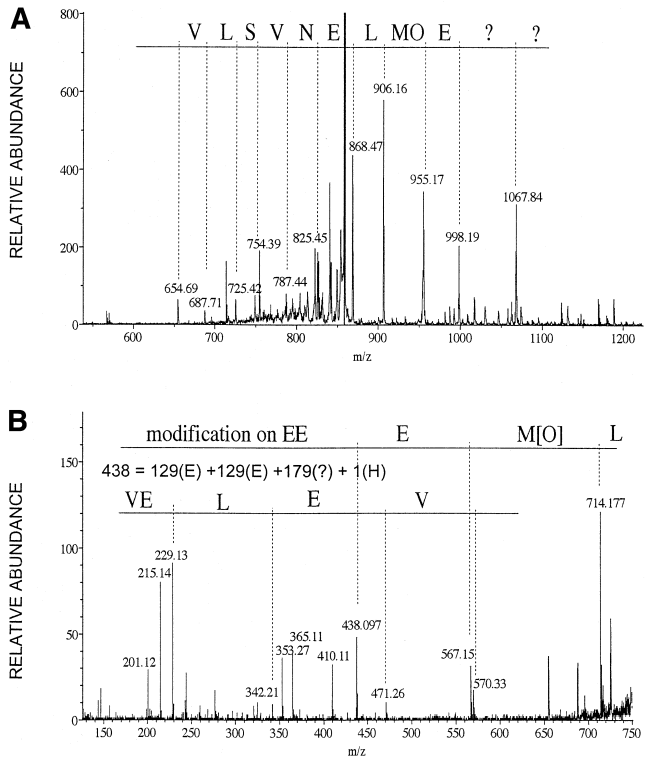

Fig. 3. MS/MS spectra of m/z = 858.164 (4+). A: The high m/z region, partial series of triply charged y fragment ions from 858.164 (4+), indicated an oxidation of the methionine residue (M4) to the corresponding sulfoxide and a divergence from the expected increase in mass through the final two N-terminal amino acids (EE). The absence of an ion at m/z = 1,041, as would be expected from continuation of the y sequence fragmentation through the unmodified peptide, is interpreted as resulting from modification of the N-terminal residue hindering normal peptide ion fragmentation. B: Ions in the low m/z region MS/MS spectrum of singly charged b series from 858.164 (4+) were consistent with the VELEV . . . sequence indicated in Fig. 2 and with the modified N-terminal peptide indicated in Fig. 5, including oxidation of the methionine residue to the corresponding sulfoxide and the 179-Da greater mass through the final two N-terminal amino acids (EE).

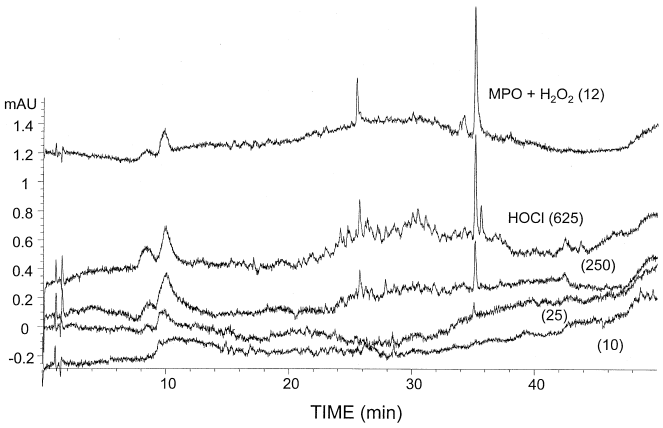

Fig. 4. Oxidation of LDL by MPO and HOCl in vitro. To 2 ml of 50 mM PBS was added 1.6 nmol of LDL and 0.02 nmol of MPO, and the mixture was incubated at 37°C for 10 min. For oxidation by the MPO-catalyzed oxidation, a total of 20 nmol of H_2O_2 (12.5 mol of H_2O_2 per mol of LDL) was added in 10 equal aliquots of 50 μl each, at 1.5-min intervals. At 37°C and in the presence of MPO, oxidation by reagent HOCl was conducted similarly by addition of 10 equally divided doses at mole ratios, the sums of which (10, 25, 250, and 625) are as indicated in the figure. Fifteen minutes after initiation of the respective oxidations, the samples were reacted with DNPH, protein was precipitated, delipidated, and digested with trypsin, and the tryptic peptides were analyzed by HPLC, as described in Materials and Methods. Detection at 365 nm only is shown. Control experiments showed no effect of the presence of MPO on product formation in oxidation with reagent HOCl, but with oxidation by H_2O_2, the product profile showed by this method was obtained only in the presence of MPO and Cl^-.

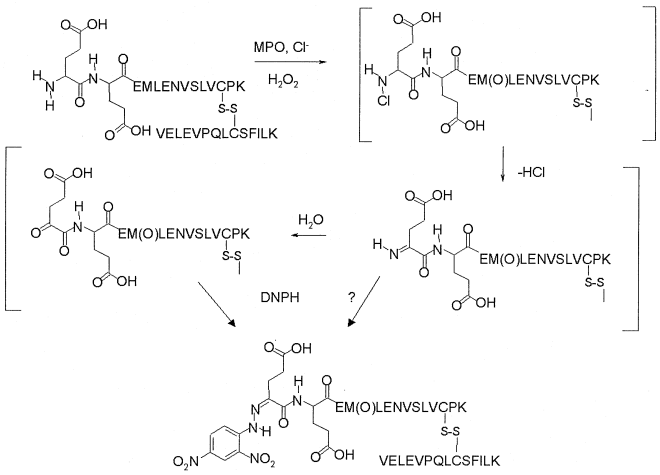

Fig. 5. MPO-catalyzed oxidation of apoB-100 in LDL. The tryptic peptides indicated by the gas-phase sequence and mass spectral analyses of the major product of MPO oxidation of LDL are indicated, as is the disulfide bond (S-S in smaller font than used to indicate serine residues in peptide sequences) linking the two peptides through their respective cysteine residues. The reaction scheme most consistent with the data available proceeds through chlorination of the terminal amine and dehydrohalogenation to the imine, with derivatization occurring directly or after hydrolysis to the corresponding ketone.

corresponding ketone before derivatization with DNPH (**Fig. 5**). The net addition of DNPH (198 Da) and loss of NH_3 (17) and of 2H (2) account for a net gain in mass of 179 Da from the unmodified sequences. Oxidation of the methionine residue to the corresponding sulfoxide accounts for a mass increase of 16, thus accounting for the observed 195-Da greater mass of the oxidized and derivatized peptide than calculated for the parent disulfide-bonded peptides sequences (21). Although other structures or mechanisms might be responsible for the data observed, none that we can envision are sufficiently reasonable to merit discussion at this time.

DISCUSSION

The clearly different product profiles evidenced by HPLC analyses of tryptic peptides from the MPO-catalyzed oxidation of intact LDL and by addition with reagent HOCl (9) indicate that HOCl is not a free intermediate in the MPO-catalyzed oxidation. Although other factors may contribute, steric restriction on access of substrate to the MPO catalytic site offers the most straightforward interpretation of the observed selectivity in product formation, without the need for more complex allosteric interactions. Studies of the structure of MPO indicate that a relatively narrow channel restricts access to the enzyme active site (22). Preferential access to the enzyme active site by the amino terminus of the apolipoprotein would contrast with the much more limited reactions observed with the C residues that were more readily oxidized by HOCl. Further, even the pendant amine and guanidino functional groups on K and R residues, respectively, appear to be less effective in accessing the enzyme active site than is the N-terminal amine of the apolipoprotein.

Monomeric substrates, including amino acids and other low molecular weight compounds, would be expected to compete successfully for MPO oxidation in free solution, and Tien (23) has observed more rapid oxidation by MPO of tyrosine as the free amino acid than in small polypeptides. The *N*-chloroamines or similar products formed by oxidation of the small molecule substrates could form secondary products by chlorine atom transfer (24) or through reactions involving imines or aldehydes formed from the chloroamines (25). However, selective oxidation by MPO of critical molecules on cell surfaces of bacteria or other target particles would provide a much greater efficiency of modification of invading pathogens than could be attained by simple production of bulk solution HOCl or other diffusible oxidants. Such a mechanism also could serve to minimize self-inactivation of MPO that would be expected from production and release of free HOCl. Previous studies have provided strong evidence that MPO can produce diffusible and volatile products of chloride oxidation, presumably HOCl and/or Cl_2 (26), but determination of the relative fluxes through these two pathways and the factors that determine the efficacies of these pathways remain to be determined, and the relative flux rates are likely to vary with types and concentrations of substrates present.

The selective oxidation of the apolipoprotein indicated by our data suggests chlorine atom transfer to the substrate amine from a bound species, in agreement with the interpretation offered by Marquez and Dunford (27) of their data on kinetics of MPO oxidation of taurine. In addition to the product selectivity directed by restricted access of substrates to the enzyme active site, such a mechanism could provide an efficient pathway for oxidation of Cl^- and a good leaving group for substrate N-chlorination. A halide-binding site that might function in such a mechanism has been described from X-ray crystal structure studies of human MPO (22), but additional studies are needed.

The mechanism proposed to explain the formation of the product observed, if valid and if applicable to the oxidation of other substances by MPO, suggests that the selective oxidation of N-terminal amino groups on cell surfaces could provide much greater efficiency in utilization of oxidants in affecting function of target pathogens, if the limited oxidative modifications are sufficient for alterations of cell functions. Previous reports that bacterial killing by MPO is enhanced by prior adherence of the enzyme to the surface of the bacteria (28) suggest mechanisms similar to those implied by our present data, but experimental tests of this working hypothesis will be needed. In addition, more specific molecular biomarkers of MPO-catalyzed oxidation of LDL and other substrates will greatly facilitate delineating the contributions of the postulated mechanisms of oxidation. More specific biomarkers are also essential for optimal evaluation and monitoring of the efficacies of therapeutic interventions.

This work was supported by grants 97G-213 and 0050530N from the American Heart Association, by grants GM44263 and T32 HL07812 from the National Institutes of Health, and by grant 2-1325 from the Welch Foundation.

Manuscript received 19 January 2001, in revised form 8 May 2001, and in re-revised form 27 July 2001.

REFERENCES

1. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo, and J. L. Witzum. 1989. Beyond cholesterol. Modifications of low density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **320:** 915–924.
2. Steinbrecher, U. P., H. Zhang, and M. Lougheed. 1990. Role of oxidatively modified LDL in atherosclerosis. *Free Radic. Biol. Med.* **9:** 155–168.
3. Berliner, J. A., M. Navab, A. M. Fogelman, J. S. Frank, L. L. Demer, P. A. Edwards, A. D. Watson, and A. J. Lusis. 1995. Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics. *Circulation.* **91:** 2488–2496.
4. Berliner, J. A., and J. W. Heinecke. 1996. The role of oxidized lipoproteins in atherogenesis. *Free Radic. Biol. Med.* **20:** 707–727.
5. Daugherty, A., J. L. Dunn, D. L. Rateri, and J. W. Heinecke. 1994. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *J. Clin. Invest.* **94:** 437–444.
6. Navab, M., S. Y. Hama, G. M. Anantharamaiah, K. Hassan, G. P. Hough, A. D. Watson, S. T. Reddy, A. Sevanian, G. C. Fonarow, and A. M. Fogelman. 2000. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J. Lipid Res.* **41:** 1495–1508.
7. Pratico, D., N. Delanty, G. DiMinno, E. Tremoli, D. Rader, S. Kapoor, J. Rokach, J. Lawson, and G. A. FitzGerald. 1998. Increased formation of distinct F_2 isoprostanes in hypercholesterolemia. *Circulation.* **98:** 2822–2828.
8. Witting, P. K., K. Pettersson, A-M. Ostlund-Lindqvist, M. Wagberg, and R. Stocker. 1999. Dissociation of atherogenesis from aortic accumulation of lipid hydro(pero)xides in Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* **104:** 213–220.
9. Yang, C-Y., Z-W. Gu, M. Yang, S-N. Lin, A. J. Garcia-Prats, L. K. Rogers, S. E. Welty, and C. V. Smith. 1999. Selective modification of apoB-100 in the oxidation of low density lipoproteins by myeloperoxidase *in vitro*. *J. Lipid Res.* **40:** 686–698.
10. Yang, C-Y., Z-W. Gu, H-X. Yang, A. M. Gotto, Jr., and C. V. Smith. 1997. Oxidative modifications of apoB-100 by exposure of low density lipoproteins to HOCl in vitro. *Free Radic. Biol. Med.* **23:** 82–89.
11. Yang, C-Y., Z-W. Gu, M. Yang, S-N. Lin, G. Siuzdak, and C. V. Smith. 1999. Identification of modified tryptophan residues in apolipoprotein B-100 derived from copper ion-oxidized low-density lipoprotein. *Biochemistry.* **38:** 15903–15908.
12. Domigan, N. M., T. S. Charlton, M. W. Duncan, C. C. Winterbourn, and A. J. Kettle. 1995. Chlorination of tyrosyl residues in peptides by myeloperoxidase and human neutrophils. *J. Biol. Chem.* **270:** 16542–16548.
13. Winterbourn, C. C., and A. J. Kettle. 2000. Biomarkers of myeloperoxidase-derived hypochlorous acid. *Free Radic. Biol. Med.* **29:** 403–409.
14. Carr, A. C., M. R. McCall, and B. Frei. 2000. Oxidation of LDL by myeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1716–1723.
15. Hawkins, C. L., and M. J. Davies. 1998. Hypochlorite-induced damage to proteins: formation of nitrogen-centred radicals from lysine residues and their role in protein fragmentation. *Biochem. J.* **332:** 617–625.
16. Hazen, S. L., and J. W. Heinecke. 1997. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J. Clin. Invest.* **99:** 2075–2081.
17. Hazell, L. J., and R. Stocker. 1993. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *Biochem. J.* **290:** 165–172.
18. Shevchenko, A., I. Chernushevich, W. Ens, K. G. Standing, B. Thomson, M. Wilm, and M. Mann. 1997. Rapid 'de novo' peptide sequencing by a combination of nanoelectrospray, isotopic labeling and a quadrupole/time-of-flight mass spectrometer. *Rapid Commun. Mass Spectrom.* **11:** 1015–1024.
19. Gerlich, D. 1992. State-selected and state-to-state ion molecular reaction dynamics. 1. Experiment. *In* Advances in Chemical Physics Series. Vol. LXXII. John Wiley & Sons, New York.
20. Krutchinsky, A. N., W. Zhang, and B. T. Chait. 2000. Rapidly switchable matrix-assisted laser desorption/ionization and electrospray quadrupole-time-of-flight mass spectrometry for protein identification. *J. Am. Soc. Mass Spectrom.* **11:** 493–504.
21. Yang, C-Y., T. W. Kim, S. A. Weng, B. Lee, M. Yang, and A. M. Gotto, Jr. 1990. Isolation and characterization of sulfhydryl and disulfide peptides of human apoB-100. *Proc. Natl. Acad. Sci. USA.* **87:** 5523–5527.
22. Fiedler, T. J., C. A. Davey, and R. E. Fenna. 2000. X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 Å resolution. *J. Biol. Chem.* **275:** 11964–11971.
23. Tien, M. 1999. Myeloperoxidase-catalyzed oxidation of tyrosine. *Arch. Biochem. Biophys.* **367:** 61–66.
24. Thomas, E. L., and D. B. Learn. 1990. Myeloperoxidase-catalyzed oxidation of chloride and other halides: The role of chloramines. *In* Peroxidases in Chemistry and Biology. J. Everse, K. E. Everse, and M. B. Grisham, editors. CRC Press, Boca Raton, FL. 83–103.
25. Hazen, S. L., F. F. Hsu, A. d'Avignon, and J. W. Heinecke. 1998. Human neutrophils employ myeloperoxidase to convert α-amino acids to a battery of reactive aldehydes: a pathway for aldehyde generation at sites of inflammation. *Biochemistry.* **37:** 6864–6873.
26. Harrison, J. E., and J. Schultz. 1976. Studies on chlorinating activity of myeloperoxidase. *J. Biol. Chem.* **251:** 1371–1374.
27. Marquez, L. A., and H. B. Dunford. 1994. Chlorination of taurine by myeloperoxidase. *J. Biol. Chem.* **269:** 7950–7956.
28. Hurst, J. K. 1990. Myeloperoxidase: active site structure and catalytic mechanisms. *In* Peroxidases in Chemistry and Biology. J. Everse, K. E. Everse, and M. B. Grisham, editors. CRC Press, Boca Raton, FL. 37–62.