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Pyridoxamine, an Inhibitor of Advanced Glycation Reactions, Also Inhibits Advanced Lipoxidation Reactions

MECHANISM OF ACTION OF PYRIDOXAMINE*

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Maillard or browning reactions lead to formation of advanced glycation end products (AGEs) on protein and contribute to the increase in chemical modification of proteins during aging and in diabetes. AGE inhibitors such as aminoguanidine and pyridoxamine (PM) have proven effective in animal model and clinical studies as inhibitors of AGE formation and development of diabetic complications. We report here that PM also inhibits the chemical modification of proteins during lipid peroxidation (lipoxidation) reactions in vitro, and we show that it traps reactive intermediates formed during lipid peroxidation. In reactions of arachidonate with the model protein RNase, PM prevented modification of lysine residues and formation of the advanced lipoxidation end products (ALEs) N°-(carboxymethyl)lysine, N°-(carboxyethyl)lysine, malondialdehyde-lysine, and 4-hydroxynonenal-lysine. PM also inhibited lysine modification and formation of ALEs during copper-catalyzed oxidation of low density lipoprotein. Hexanoic acid amide and nonanedioic acid monoamide derivatives of PM were identified as major products formed during oxidation of linoleic acid in the presence of PM. We propose a mechanism for formation of these products from the 9- and 13-oxo-decadienoic acid intermediates formed during peroxidation of linoleic acid. PM, as a potent inhibitor of both AGE and ALE formation, may prove useful for limiting the increased chemical modification of tissue proteins and associated pathology in aging and chronic diseases, including both diabetes and atherosclerosis.

Aging and age-related diseases, such as diabetes, atherosclerosis, and neurodegenerative diseases, are characterized by increases in oxidative chemical modification of tissue proteins (1, 2). Some oxidation products, e.g. methionine sulfoxide and o-tyro sine, are formed by direct oxidation of amino acids in proteins. Glycoxidation products or advanced glycoxidation end products (AGEs), such as the cross-links pentosidine and vesperlysin es (3, 4), are produced by secondary modification of proteins by products of carbohydrate oxidation. Advanced lipoxidation end products (ALEs), including malondialdehyde (MDA) and hydroxynonenal (HNE) adducts to lysine (MDA-Lys, HNE-Lys) (5) and pyrroles (6), are formed during lipid peroxidation reactions, whereas other compounds, such as N°-(carboxymethyl)lysine (CML) and N°-(carboxyethyl)lysine (CEL), are formed during both glycoxidation and lipoxidation reactions (7). Age-adjusted levels of pentosidine and CML are increased during chronic hyperglycemia and correlate with the severity of renal, retinal, and vascular complications in diabetes (8). Inhibitors of AGE formation, such as aminoguanidine (9), tenilsetam (10), OPB-9195 (11), and pyridoxamine (PM) (12, 13), also retard the development of diabetic complications in animal models, and aminoguanidine and PM are being evaluated in clinical trials for treatment of diabetic nephropathy. Although AGE inhibitors vary widely in structure, they are all nucleophilic compounds and are thought to function by trapping reactive carbonyl intermediates in AGE formation. Because they are relatively nonspecific nucleophiles, AGE inhibitors also affect other oxidative chemistry, e.g. the formation of ALEs. Aminoguanidine, for example, inhibits chemical modification of proteins during lipid peroxidation reactions (14) and inhibits metal-catalyzed oxidation of low density lipoprotein (LDL) and uptake of oxidized LDL into macrophages via the scavenger receptor (15).

The AGE inhibitor PM was introduced by Hudson and colleagues (12, 13) as an inhibitor of AGE formation from Amadori compounds, the first stable sugar-protein adducts formed during glycation of protein. In animal model studies, PM also inhibited AGE formation and retarded the development of nephropathy in streptozotocin-induced diabetic rats (16). However, the renal protection was accompanied by a significant reduction in hypertiglyceridemia, and there were strong correlations between plasma triglyceride concentration and the levels of CML and CEL, fluorescence, and cross-links in skin collagen. These observations suggested that, in addition to its AGE-inhibitory activity, PM might also affect lipid homeostasis and 9,11-oxo-decadienoic acid; LA, linoleic acid; LC-ESI-MS, liquid chromatography-electrospray ionization-mass spectrometry; LDL, low density lipoprotein; MDA, malondialdehyde; MDA-Lys, malondialdehyde-lysine, Schiff base adduct; PM, pyridoxamine; PUFAs, polyunsaturated fatty acid; RNase, bovine pancreatic ribonuclease A; RP-HPLC, reverse-phase high performance liquid chromatography; TBARS, thiorbarbituric acid-reactive substances; TNBS, trinitrobenzensulfonic acid; MS, mass spectrometry; GC/MS, gas chromatography/mass spectrometry; ALEs, advanced lipoxidation end products.

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‡ The abbreviations used are: AGE, advanced glycation end product; AA, arachidonic acid; CER, N°-(carboxymethyl)lysine; CML, N°-(carboxyethyl)lysine; DTPA, diethylenetriaminopentaaetic acid; HNE-Lys, Michael addition adduct of lysine to 4-hydroxynonenal; KODE, keto-octadecadienoic acid (9-oxo-11,13-octadecadienoic acid or 13-oxo-
lipid-dependent chemical modification of protein in vivo. In this paper we describe experiments in vitro on the effects of PM on the chemical modification of proteins during lipid peroxidation reactions, and we show that PM is a potent inhibitor of modification of lysine by peroxidizing lipids, both in a model protein-lipid system and during copper-catalyzed oxidation of LDL. We also identify the major products formed on reaction of linoleic acid (LA) with PM under autokitative conditions, the hexanoic acid amide and nonanecic acid monoamide derivatives of PM, and we propose a mechanism for PM inhibition of chemical modification of proteins during lipoxidation reactions. Our results provide insight into the mechanism of action of PM as an AGE and ALE inhibitor, and we suggest that PM may be useful for inhibiting the increased chemical modification of tissue proteins, whether derived from carbohydrate or lipids, in diabetes, atherosclerosis, and other chronic diseases.

MATERIALS AND METHODS

Chemicals—Arachidonic acid (AA), LA, oleic acid, palmitic acid, PM(HCl), piridoxal(1H)-, pyridoxine(1H)-, ribonuclelease, N-ethylmaleimide, N-acetyl-L-lysine, N-acetyl-L-leucine, N-acetyl-L-propyl, methyl linoleate, and nonanecic (azelaid acid) mono-methyl ester were purchased from Sigma. Boron trichloride was purchased from Pierce and heptafluorobuturylic acid from Acros Chemicals (Atlanta, GA). [2H4]- and [2H8]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards ([1H]- and [2H]-lysine were from Isotec (Miamisburg, OH). Deuterated and natural abundance internal standards. 

Linoleate was converted to methyl linoleate using 0.5 ml of dry ether added dropwise over an hour while the reaction was carried out on a Hewlett-Packard model 6890 gas chromatograph/5970 mass spectrometer (ESI-LC/MS), as described below.

Other Methods—N-Hexanoyl-pyridoxamine was synthesized according to D’Alelio and Reid (23). Briefly, 0.3 g of PM in 0.3 ml of deionized water was added to 0.3 g of azelaic acid mononyl ester and heated at 140 °C for 10 h. The brown reaction mixture was dried in vacuo and reconstituted in deionized water/acetic acid/1:1, v/v. N-Nonanedioyl-PM was identified and isolated by RP-HPLC with detection by absorbance and mass spectrometry (ESI-LC/MS), as described below.

N-Acyl-PM derivatives were hydrolyzed in 2 N HCl for 4 h at 95 °C, releasing free PM and carboxylic acids. The hydrolysate was dried in vacuo, and the resulting free carboxylic acids (hexanoic and nonanecic acids) were analyzed by GC/MS as their propyl and dimethyl esters, respectively. Esterification was performed in 1 ml of 1 N HCl in n-propyl alcohol or methanol at 65 °C for 1 h. The esters were extracted into 2 ml of hexane/water (2:1, v/v). Following vortexing and centrifugation, the hexane phase was analyzed by GC/MS, as described below.

The aqueous phase, containing PM, was dried in vacuo, and the PM was acetylated by reaction with acetic anhydride: 3 ml of acetyldride (1.1, v/v) for 2 h at room temperature. Following evaporation in vacuo, the product was reconstituted in methylene chloride for GC/MS analysis.

Mass Spectrometry—ESI-LC-MS was carried out in the positive ion mode on a VG (Manchester, UK) Trio-3 triple quadrupole mass spectrometer equipped with a Hewlett-Packard (Palo Alto, CA) series 1100 HPLC system and a Keystone (Bellefonte, PA) Aquasil C-18 microbore column. The solvent system consisted of 0.1% heptafluorobutyric acid (solvent A) and acetonitrile (solvent B), flow rate 0.6 ml/min. The gradient was as follows: 0–1 min, 95% A; 1–9 min, 5% A; hold for 2 min; 9–10 min, 5% A; hold for 4 min; 10–12 min, 100% B; hold for 10 min. GC/MS was carried out on a Hewlett-Packard model 6890 gas chromatograph/5970 mass selective detector, using a 30-meter HP-5MS (5% phenyl methyl silicone) capillary column (Restek, Bellefonte, PA). The temperature program for analyses of PM and carboxylic acid esters was as follows: initial temperature 75 °C, 6 °C/min ramp to 110 °C, 10 °C/min ramp to 180 °C hold 5 min, 12 °C/min ramp to 270 °C/min, hold 5 min. Fast atom
RESULTS

Reaction of PM with PUFAs—To study the interaction of PM with products of lipid peroxidation, PM was incubated with oleate or PUFAs in phosphate buffer at physiological pH, relying on endogenous metal ions in the phosphate buffer to catalyze PUFA oxidation. As shown in Fig. 1A, PM incubated alone or in the presence of oleate was recovered unchanged over the 6-day incubation period. However, in the presence of LA and AA, PM was gradually consumed, with about 60% PM loss at 6 days, based on HPLC analysis (see inset to Fig. 4). When the same samples were analyzed by the TNBS assay (Fig. 1B), 60% of the primary amino groups, measured by the TNBS assay. Data are means ± S.D. of three independent experiments.

PM Inhibition of Lipoxidative Modification of RNase by AA—The activity of PM as an inhibitor of lipoxidative modification of proteins was studied in reactions of AA with RNase. As shown in Fig. 3, CML, CEL, MDA-Lys, and HNE-Lys were formed continuously on RNase during the incubation with AA. These studies confirm previous reports on the formation of CML during lipid peroxidation reactions (18) and also identify CEL as a product of arachidonate oxidation. The decline in MDA-Lys recovered after 1 day resembles that observed during copper oxidation of LDL and is thought to result from further reaction of MDA-Lys to form other products, such as Lys-MDA-Lys (5). Addition of 1 mM PM to the AA + RNase mixture resulted in almost complete inhibition of formation of all the ALEs. Notably, PM provided potent protection against lipoxidative modification of RNase, although it was present at only 1% the concentration of AA, and 10% the concentration of lysine residues in RNase. The small increase in formation of lipoxidation products by day 6 likely reflects the fact that all of the PM had been consumed by that time, as shown in Fig. 4. Amino acid analysis indicated that there was a progressive loss of lysine during the reaction, with about 58% of lysine residues lost at the end of 6 days in the absence of PM (Fig. 5). In contrast, inclusion of PM in the incubation resulted in loss of only about 5% of lysine residues by day 6.

Inhibition of LDL Oxidation by PM—Because PM inhibited lipoxidation product formation in the AA + RNase system, we also evaluated its effectiveness as an inhibitor of lipoxidation during copper-catalyzed oxidation of LDL, a common in vitro model for studies on lipoxidative modification of proteins. PM was used at concentrations of 100 and 250 μM, comparable to the ~120 μM concentration achieved in rats treated with 1 g/liter in drinking water (2,3). At these concentrations, PM caused a 2–3-fold increase in the lag phase of LDL oxidation and an ~20% decrease in absorbance at 234 nm during the later plateau phase of the reaction (Fig. 6A). Confirming the results of experiments with AA and RNase, PM was an effective inhibitor of formation of CML, CEL, MDA-Lys, and...
HNE-Lys (Fig. 6B) during metal-catalyzed oxidation of LDL. The relative yields of the ALEs during oxidation of LDL differed significantly from yields obtained in reactions of AA with RNase, probably reflecting the heterogeneous fatty acid composition of LDL. PM was more effective in limiting formation of CML and CEL, compared with MDA-Lys and HNE-Lys (Fig. 6B), but was a potent inhibitor of the overall modification of lysine residues in oxidized LDL (Fig. 6C), with only 6% loss in the presence of PM compared with 29% in its absence.

Identification of PM Adducts—To identify products trapped by PM during PUFA oxidation, we studied the reaction of PM (1 mM) with LA, the primary PUFA in LDL. HPLC analyses of the LA + PM reaction, described in Figure 7, indicated formation of products with fluorescence (Fig. 7, B versus A) and absorbance (Fig. 7C) maxima characteristic of PM. The consumption of PM over a period of 6 days of reaction is evident, as well as the time-dependent formation of new products eluting later than PM, indicating that these adducts were more hydrophobic than PM itself. Two major fluorescent products were consistently observed, eluting at ~24 min. Based on their fluorescence intensity, these two products accounted for ~10% of the PM in the starting reaction, whereas ~65% of the starting PM was consumed by 6 days (Fig. 1). The fluorescence of the total reaction mixture was also measured at 6 days, to establish that fluorescent reaction products were not binding irreversibly to the C-18 column. These measurements confirmed a significant ~50% decrease in fluorescence during the reaction, consistent with the overall loss of fluorescence observed in the HPLC analyses in Figure 7C. This observation suggested that the PM derivatives were less fluorescent than PM itself.

Semi-preparative RP-HPLC analysis (Fig. 7C) showed that a number of additional products with absorbance maxima at 294 nm were also detected after 6 days of reaction. Those eluting after about 23 min (indicated by an asterisk) had absorbance maxima below 260 nm and were also formed from LA, in the absence of PM. However, products eluting between 19 and 22 min had extracted absorbance maxima at 294 nm (photodiode array detector), consistent with the presence of the intact pyridine ring. The peaks eluting in this region of the chromatogram were pooled and subjected to direct injection ESI-MS. Major products identified had m/z values of 267, 305, 339, 377, and 479 (Fig. 8A); none of these were present in incubations of LA or PM alone. Assignment of the various species to particular chromatographic peaks was accomplished by collecting individual peaks by semi-preparative RP-HPLC (Fig. 8B) and analysis by direct injection ESI-MS. Once peak assignments were made, the kinetics of formation of the three most abundant products (267, 305, and 339) were determined by RP-HPLC (Fig. 8C). The yields of all three compounds increased with time, with products 267 and 339 consistently forming in the highest yields. These products were chosen for further characterization.

Product Characterization—Products 267 and 339 were identified as the hexanoic acid amide and nonanedioic (azelaic) acid monoamide derivatives of PM (24). The hexanoic acid amide derivative of lysine has been identified previously by Kato et al. (25, 26) in reactions of linoleic acid hydroperoxides with N\textsuperscript{\textbeta}-
blocked lysine, was shown to be formed during copper-catalyzed oxidation of LDL, and was identified in the vascular wall by immunohistochemical methods. Data on product 339 are therefore presented in more detail here. Products 267 and 339 were isolated by semi-preparative RP-HPLC. Based on absorbance area units on HPLC, both products yielded PM (\(\text{PM} \approx 90\%\)) on acid hydrolysis (2 N HCl, 3 h at 95 °C) (shown for product 339 in Fig. 9, A versus B). The fluorescence response of these products on HPLC analysis was 30% that of PM, consistent with the loss of PM fluorescence during reaction of LA with PM (Fig. 7, B versus A). Neither product contained a free amino group, based on lack of reactivity with TNBS, consistent with the loss of TNBS reactivity during lipid peroxidation reactions (Fig. 18).

The hexanoic acid amide and nonanedioic acid monoamide derivative of PM were prepared synthetically, as described under "Materials and Methods." Analysis of the synthetic products by RP-HPLC-ESI-MS confirmed that they co-eluted with products 267 and 339 obtained from the reaction mixture and

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**Fig. 6.** Pyridoxamine does not prevent copper-catalyzed oxidation of LDL but does inhibit ALE formation and lysine loss. LDL (50 \(\mu\)g/ml) was incubated at 37 °C in phosphate-buffered saline alone (□) or in the presence of 5 \(\mu\)M Cu\(^{2+}\) (●) or with 5 \(\mu\)M Cu\(^{2+}\) and 100 \(\mu\)M (∆) or 250 \(\mu\)M (▲) PM. A, conjugated diene formation was monitored at 234 nm. At the end of the incubation ~1 mg of protein was removed, reduced with NaBH\(_4\), dialedyzed, delipidated, and then analyzed for CML, CEL, MDA-Lys, and HNE-Lys content by selected ion monitoring (SIM)-GC/MS (B) and lysine loss (C) by amino acid analysis. Results are mean and range for analysis of two independent pools of LDL and are representative of two separate experiments.

**Fig. 7.** Pyridoxamine is consumed, and fluorescent and absorbant products are formed during oxidation of linoleic acid. PM (1 mM) was incubated with LA (5 mM) in 0.2 M sodium phosphate buffer, pH 7.4, for 6 days at 37 °C. A and B, aliquots of the aqueous phase were analyzed by analytical RP-HPLC at various times using fluorescence detection with pyridoxal added as an internal standard, just prior to chromatography. Products indicated by brackets were consistently formed in reaction mixtures in the presence of PM. C, semi-preparative chromatographic analysis of a sample at 6 days, monitoring absorbance at 294 nm. The brackets in C indicate the region pooled from several semi-preparative column runs and were used for mass spectrometry analysis. The * indicates products produced during LA oxidation in the absence of PM.

**Fig. 8.** Identification and kinetics of formation of pyridoxamine adducts in reactions of pyridoxamine with linoleic acid. A, products formed after 6 days of reaction and eluting between 19 and 22 min (Fig. 7D) were analyzed by direct injection ESI-MS. Five major products were identified, with \(m/z = 267, 305, 323, 339,\) and 479. B, semi-preparative RP-HPLC (absorbance detection), identifying major products with \(m/z = 339, 267,\) and 305, in order of elution. C, kinetics of formation of 267 (●), 339 (■), and 305 (▲), based on their area ratios to pyridoxal, added as internal standard. The data shown are the mean and S.D. from three independent experiments.
yielded identical molecular ions by ESI-MS analysis. Acid hydrolysis (2 M HCl, 2 h, 95 °C) of products 267 and 339 (both synthetic and from reaction mixtures) also yielded PM by HPLC and either hexanoic or nonanedioic acid by GC/MS as the propyl or dimethyl ester, respectively (shown for reaction product 339 in Fig. 9, C and D). Both PM and the respective carboxylic acid were formed in approximately equal proportions (>90% yield), confirming the identity of products 267 and 339.

Product 305 has not yet been isolated in sufficient amounts for complete characterization. Based on its molecular formula, determined by high resolution fast atom bombardment-MS, product 305 is a 9-carbon mono-oxygenated adduct to PM, possibly an epoxy or hydroxy acid derivative of PM. It was not formed by reaction of HNE with PM in phosphate buffer (data not shown). Product 479 was a minor reaction product. Its molecular weight indicates that it is formed by adduction of PM to an intact, dioxo-derivative of LA. Further characterization of these and other reaction products is in progress.

**DISCUSSION**

**PM Inhibition of Advanced Lipoxidation Reactions**—PM was originally described as an AGE inhibitor and, like other AGE inhibitors, is thought to act as a nucleophilic trap for reactive carbonyl intermediates in AGE formation. Reactive carbonyl compounds are also involved in the formation of ALEs, so it seemed likely that this compound would also inhibit chemical modification of proteins during lipid peroxidation reactions. Indeed, PM was a potent inhibitor of chemical modification of both RNase and LDL during lipid peroxidation reactions, inhibiting both the modification of lysine residues and the formation of specific lipoxidation products, CML, CEL, MDA-Lys, and HNE-Lys. Some weak antioxidant activity, a general characteristic of phenolic compounds, was also observed, as judged by a marginal effect of PM on the kinetics of oxidation of linoleate and LDL. However, the gradual solubilization of the PUFA and chemical modification of PM in reactions in either the absence or presence of protein, the formation of TBARS and decomposition of LA (Fig. 2), as well as the formation of conjugated dienes during oxidation of LDL (Fig. 6) document that lipid peroxidation occurred, even in the presence of PM. The overall observations were consistent with the hypothesis that PM acts as a sacrificial nucleophile, trapping reactive intermediates in lipoxidation (and glycoxidation) reactions. The involvement of the amino group of PM in inhibition of ALE formation was confirmed by the loss of TNBS reactivity in reactions of PM with oxidizing PUFA (Fig. 1B).

The fact that PM protects against modification of lysine residues and formation of lipoxidation products in RNase, despite the 100-fold excess of PUFA and the 10-fold higher concentration of lysine residues on RNase (Figs. 3 and 4), illustrates its efficiency in trapping reactive carbonyl intermediates. The efficiency of PM in protecting lysine amino groups suggests either that only a fraction of lipid peroxidation products participate in chemical modification of protein or that PM intercepts critical, early lipid peroxidation products, preventing their decomposition to a broader range of shorter-chain reactive carbonyl compounds. Consistent with the latter hypothesis, none of the anticipated ALE derivatives of PM, e.g. carboxymethyl-PM, carboxyethyl-PM, MDA-PM, or HNE-PM, were identifiable in the PM + AA reaction mixture, based on scanning for their molecular weight by LC-ESI-MS. Thus, it appears that, by trapping early intermediates in glycoxidation and lipoxidation reactions, PM protects against the formation of a range of later precursors of ALEs on proteins.

The inhibition of lipoxidation in the RNase model system suggested that PM would exert similar effects during copper-catalyzed oxidation of LDL. As shown in Fig. 6, despite the high rate of lipoprotein oxidation catalyzed by 5 μM Cu²⁺ in this
Pyridoxamine Inhibits Lipoxidation Reactions

Mechanism of Action of PM—PM is unique among current inhibitors of advanced glycation reactions because of its characteristic fluorescence spectrum. Thus, the carboxyl intermediates trapped by PM are not only amenable to RP-HPLC analysis but are also readily identifiable by fluorescence detection (Fig. 7). These features of PM, combined with previous work of Kato et al. (25, 26), facilitated the identification of the hexanoic acid amide and nonanedioic acid monoamide derivatives of PM. These compounds are clearly formed by addition of PM to the 9- and 13-carbons of LA, followed by oxidative cleavage of the 9-10 or 12-13 carbon-carbon bonds (Scheme I). In support of this mechanism, KODEs have been identified as products of both non-enzymatic (27, 28) and enzymatic (29, 30) peroxidation of LA and have also been detected by chemical methods in atherosclerotic plaque (31). Thiols compounds are known to react directly with KODEs, whereas their reaction with hydroperoxyoctadecadienoic acids requires autoxidizing conditions (Fe$^{3+}$ and O$_2$) (16, 17). PM also fails to react with lipid peroxides (prepared from LA using soybean lipoxygenase) under anti-oxidative conditions (anaerobic incubation in the presence of DTPA; data not shown.). Thus, PM may function as a surrogate, non-thiol nucleophile, trapping and inactivating KODE intermediates and thereby inhibiting further chemical modification of proteins by peroxidizing lipids.

Concluding Remarks—Based on the proposed mechanism of formation of PM adducts, the hexanoic derivative of lysine is likely to be formed from any α-6 PUFA. A number of other mono- and dicarboxylic acid amide derivatives should also be formed on oxidation of other families of PUFA. Analysis of the amide content of tissue proteins should therefore provide insight into both the origin of lipid-derived chemical modifications of proteins and their contribution to the overall chemical modification of proteins in vivo. These non-enzymatic lipid adducts to protein promote protein-membrane interactions, affecting the endocytosis, subcellular transport, compartmentalization, and catalysis of lipidized proteins. These other lipid adducts may also contribute to the accumulation of lipofuscin in postmitotic tissues during aging and to the accumulation of lipids and lipoproteins in foam cells in the vascular wall in atherosclerosis. We are encouraged by the possibility that inhibition of lipidoxidative modification of proteins, using compounds such as PM, may provide a route for limiting age-dependent and disease-related damage to proteins by both glycoxidation and lipoxidation reactions and may be useful for treatment of diseases involving hyperlipidemia and/or oxidative stress.

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