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Protein-bound acrolein: Potential markers for oxidative stress

(covalent modification of protein/antibody/atherosclerosis)

KOJI UCHIDA*†, MASAMICHI KANEMATSU*, KENSUKE SAKAI*, TSUKASA MATSUDA‡, NOBUTAKA HATTORI§, YOSHIKUNI MIZUNO§, DAISUKE SUZUKI¶, TOSHIO MIYATA¶, NORIKO NOGUCHI‖, ETSUO NIKI‖, AND TOSHIHIKO OSAWA*

*Laboratory of Food and Biodynamics and ‡Laboratory of Molecular Bioregulation, Nagoya University Graduate School of Bioagricultural Sciences, Nagoya 464-8601, Japan; §Department of Neurology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo, Tokyo 113, Japan; ¶Institute of Medical Sciences and Department of Medicine, Tokai University School of Medicine, Isehara 259-11, Japan; and ‖Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Tokyo 153, Japan

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ABSTRACT Acrolein (CH_2═CH—CHO) is known as a ubiquitous pollutant in the environment. Here we show that this notorious aldehyde is not just a pollutant, but also a lipid peroxidation product that could be ubiquitously generated in biological systems. Upon incubation with BSA, acrolein was rapidly incorporated into the protein and generated the protein-linked carbonyl derivative, a putative marker of oxidatively modified proteins under oxidative stress. To verify the presence of protein-bound acrolein *in vivo*, the mAb (mAb5F6) against the acrolein-modified keyhole limpet hemocyanin was raised. It was found that the acrolein-lysine adduct, N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine, constitutes an epitope of the antibody. Immunohistochemical analysis of atherosclerotic lesions from a human aorta demonstrated that antigenic materials recognized by mAb5F6 indeed constituted the lesions, in which intense positivity was associated primarily with macrophage-derived foam cells and the thickening neointima of arterial walls. The observations that (*i*) oxidative modification of low-density lipoprotein with Cu^{2+} generated the acrolein-low-density lipoprotein adducts and (*ii*) the iron-catalyzed oxidation of arachidonate in the presence of protein resulted in the formation of antigenic materials suggested that polyunsaturated fatty acids are sources of acrolein that cause the production of protein-bound acrolein. These data suggest that the protein-bound acrolein represents potential markers of oxidative stress and long-term damage to protein in aging, atherosclerosis, and diabetes.

Lipid peroxidation has been implicated in the pathogenesis of numerous diseases including atherosclerosis, diabetes, cancer, and rheumatoid arthritis, as well as in drug-associated toxicity, postischemic reoxygenation injury, and aging (1). In atherosclerosis, the oxidation of circulating low-density lipoprotein (LDL) and their increased uptake by the scavenger receptor is thought to promote the deposition of lipid-laden macrophages in the vascular wall, leading to fatty streaks that precede the development of plaque (2). Lipid peroxidation proceeds by a free radical chain reaction mechanism and yields lipid hydroperoxides as major initial reaction products. Subsequently, decomposition of lipid hydroperoxides generates a number of breakdown products that display a wide variety of damaging actions (3). There is increasing evidence that aldehydes among them are causally involved in most of the pathophysiological effects associated with oxidative stress in cells and tissues. Aldehydes in lipid peroxidation have been considered as the end products in lipid peroxidation. However, they are still active and exhibit facile reactivity with various biomolecules, including proteins and phospholipids, generating stable products at the end of a series of lipid peroxidation reactions (3–6) that are thought to contribute to the pathogenesis of various diseases.

Acrolein, an unpleasant and troublesome by-product of overheated organic matter, occurs as a ubiquitous pollutant in the environment, e.g., incomplete combustion of plastic materials, cigarette smoking, and overheating frying oils. Acrolein is also a metabolite formed in the biotransformation of allyl compounds and the widely used anticancer drug cyclophosphamide. Its high reactivity indeed makes acrolein a dangerous substance for the living cell. Among all α,β-unsaturated aldehydes including 4-hydroxy-2-nonenal, acrolein is by far the strongest electrophile and, therefore, shows the highest reactivity with nucleophiles, such as the sulfhydryl group of cysteine, imidazole group of histidine, and amino group of lysine (3). Acrolein undergoes nucleophilic addition at the double bond (C—3) to form a secondary derivative with retention of the aldehyde group, resulting in the formation of the Michael addition-type acrolein-amino acid adducts. It has been shown that acrolein modifies lysine and histidine residues of human serum albumin (7) and α-1-proteinase (8). Pocker and Janjic (9) showed that acrolein modified the histidine residues of carbonic anhydrase, and, employing the N^{α}-acetylhistidine or imidazole model, they identified as the predominant adduct formed, 3-(*N*-imidazole)propanal. Although it has been proposed that, upon reaction of acrolein with amino groups, acrolein forms β-substituted propanals (R—NH—CH_2—CH_2—CHO) and Schiff's base (R—NH—CH_2—CH_2—CH═N—R) (3), an acrolein-lysine adduct, N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine), has been recently identified as the major product (K.U., M.K., Y. Morimitsu, T.O., N.N., and E. Niki, unpublished data).

In the present study, using a mAb raised against the acrolein-modified protein, we show evidence that acrolein is a lipid peroxidation product that is accumulated in the form covalently bound to proteins *in vivo*.

EXPERIMENTAL PROCEDURES

Materials. N^{α}-Acetyl-L-lysine, N^{α}-acetyl-L-histidine, acrolein, and BSA were obtained from Sigma. Keyhole limpet hemocyanin (KLH) was obtained from Pierce. Horseradish peroxidase-linked anti-rabbit IgG Ig and enhanced chemilu-

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Abbreviations: FDP-lysine, N^{ε}-(3-formyl-3,4-dehydropiperidino)lysine; LDL, low-density lipoprotein; KLH, keyhole limpet hemocyanin.

†To whom reprint requests should be addressed. e-mail: uchidak@agr.nagoya-u.ac.jp.

minescence Western blot analysis detection reagents were obtained from Amersham.

Preparation of Acrolein-N^{α}-Acetyllysine and Acrolein-N^{α}-Acetylhistidine Adducts. The reaction mixture (10 ml) contained 50 mM acrolein and 100 mM N^{α}-acetyllysine or 100 mM N^{α}-acetylhistidine in 50 mM sodium phosphate buffer (pH 7.2). After incubation for 24 h at 37°C, the acrolein-N^{α}-acetyllysine adducts were analyzed and purified with a reverse-phase HPLC by using a Develosil ODS-HG-5 column (8 × 250 mm) (Nomura Chemicals, Seto, Japan) equilibrated in a solution of 5% methanol in 0.1% trifluoroacetic acid, flow rate being 2.5 ml/min. The elution profiles were monitored by absorbance at 227 nm. The *N*-formylethylated histidine adduct was isolated with a reverse-phase HPLC using a Develosil ODS-HG-5 column equilibrated in a solution of 0.1% heptafluorobutyric acid, flow rate being 2.5 ml/min. The elution profiles were monitored by absorbance at 215 nm. The chemical structures of N^{α}-acetyl-FDP-lysine and N^{α}-acetyl-N^{im}-propanalhistidine have been characterized by ^{1}H- and ^{13}C-NMR and liquid chromatography-MS.

Protein Carbonyl. An aliquot (0.5 ml) of protein samples was treated with an equal volume of 0.1% (wt/vol) 2,4-dinitrophenylhydrazine in 2 M HCl and incubated for 1 h at room temperature. This mixture was treated with 0.5 ml of 20% trichloroacetic acid (wt/vol, final concentration), and after centrifugation, the precipitate was extracted three times with ethanol/ethyl acetate (1:1, vol/vol). The protein sample was then dissolved with 2 ml of 8 M guanidine hydrochloride, 13 mM EDTA, and 133 mM Tris solution (pH 7.4). UV absorbance was measured at 365 nm. The results were expressed as mol of 2,4-dinitrophenylhydrazine/protein (mol/mol) based on an average absorptivity of 21.0 mM^{-1}/cm^{-1}.

Amino Acid Analysis. Changes in the amino acid composition of protein were assessed by amino acid analysis. An aliquot (0.1 ml) of LDL incubated in the absence and presence of acrolein or Cu^{2+} was treated with 10 mM EDTA (10 μl), 1 M NaOH (10 μl), and 100 mM $NaBH_4$ (10 μl). After incubation for 1 h at 37°C, the mixture was treated with 1 M HCl (30 μl), evaporated to dryness, and then hydrolyzed *in vacuo* with 6 M HCl for 24 h at 105°C. The hydrolysates were concentrated and dissolved with 50 mM sodium phosphate buffer (pH 7.4). The amino acid analysis was performed with a JEOL JLC-500 amino acid analyzer equipped with a JEOL LC30-DK20 data analyzing system.

***In Vitro* Peroxidation of LDL.** LDL was separated from the plasma of healthy donor by ultracentrifugation as described in the literature (10) within a density cut-off of 1.019 to 1.063 g/ml and then dialyzed with cellulose membranes in PBS (pH 7.4) containing 100 μM EDTA. It was sterilized with a Mille-GV filter (Millipore) after dialysis. The protein concentration of LDL was measured using the bicinchoninic acid protein assay reagent (Pierce). LDL was dialyzed in PBS (pH 7.4) to remove EDTA. The oxidation of LDL (0.5 mg protein/ml) by 5 μM Cu^{2+} was carried out at 37°C under air in PBS (pH 7.4).

***In Vitro* Modification of BSA.** The iron-catalyzed oxidation of arachidonate in the presence of BSA was performed by incubating BSA (1 mg/ml) with 2 mM arachidonate in the presence of either 10 μM Fe^{2+}, 1 mM ascorbate, or 10 μM Fe^{2+} and 1 mM ascorbate in 0.1 ml of 0.1 M sodium phosphate buffer (pH 7.4) under atmospheric oxygen.

Antibody Preparation. *(i) Polyclonal Antibody.* Polyclonal antiserum against acrolein-modified proteins was raised by immunizing a New Zealand White rabbit with KLH that had been treated with acrolein. The acrolein-modified KLH immunogen was prepared by the reaction of 1 mg KLH with 10 mM acrolein in 1 ml of 50 mM sodium phosphate buffer, pH 7.2, for 24 h at 37°C. The ratio of acrolein to amino groups of KLH was therefore 1:50. The acrolein-modified KLH (1 mg/ml) was emulsified in the same volume of Freund's complete adjuvant and incubated intradermally into several sites in New Zealand White rabbits. After 4, 6, 8, and 11 weeks, the s.c. booster injections in Freund's incomplete adjuvant were repeated. Antibody response was monitored by immunoblots using acrolein-modified BSA as the antigen.

(ii) mAb. Female BALB/c mice were immunized three times with the acrolein-treated KLH. Spleen cells from the immunized mice were fused with P3/U1 murine myeloma cells and cultured in hypoxantine/amethopterin/thymidine selection medium. Culture supernatants of the hybridoma were screened using an ELISA, employing pairs of wells of microtiter plates on which were absorbed acrolein-treated BSA, oxidized LDL, and native LDL as antigen (1 μg of protein per well). After incubation with 100 μl of hybridoma supernatants, and with intervening washes with Tris-buffered saline, pH 7.8, containing 0.05% Tween 20 (Tris-buffered saline-Tween), the wells were incubated with alkaline phosphatase-conjugated goat anti-mouse IgG, followed by a substrate solution containing 1 mg/ml *p*-nitrophenyl phosphate. Hybridoma cells corresponding to supernatants that were positive on both acrolein-modified BSA and oxidized LDL and negative on native LDL were then cloned by limiting dilution. After repeated screening, four clones were obtained. Among them, clone 5F6 showed the most distinctive recognition of both acrolein-modified BSA and oxidized LDL.

ELISA. The competitive and noncompetitive ELISA were performed as described (11).

Immunohistochemistry. For indirect immunohistochemical staining, human arterial tissues obtained at autopsy were cut into 5-μm sections, mounted on the slides coated with 3-aminopropyltriethoxy silane, deparaffined, rehydrated in distilled water, incubated with pronase (0.5 mg/ml) (Dako), and then blocked in 4% skim milk for 2 h. The sections were incubated with either anti-acrolein-modified KLH polyclonal antibody, mAb5F6, or anti-CD68 mouse monoclonal IgG (2 μg/ml) (Dako) overnight in humid chambers at room temperature. The sections were washed and incubated with 1:100 diluted swan anti-rabbit or rabbit anti-mouse IgG conjugated with peroxidase (Dako) for 2 h at room temperature, followed by detection with 3,3′-diaminobenzidine solution containing 0.003% H_2O_2. Competition experiments to confirm the specificity of immunostaining were also performed with the antibody that was preincubated for 4 h at 37°C with an excess of N^{α}-acetyl-FDP-lysine. Nonimmune rabbit or mouse IgG was used as a negative control.

RESULTS

Covalent Binding of Acrolein to Protein. The covalent binding of acrolein to protein was examined by generation of protein-linked carbonyl groups and changes in amino acid composition. As shown in Fig. 1, exposure of BSA (1 mg/ml) to 1 mM acrolein resulted in a time-dependent increase in carbonyl formation. The incorporation of acrolein into the protein was accompanied by selective loss of amino acid residues. When the protein (1 mg/ml) was treated with 1 mM acrolein for 24 h at 37°C, 26 molecules of lysine residues and 8 molecules of histidine residues per molecule of protein were lost. The sum of the amounts of lysine and histidine residues lost (34 mol/mol protein) is almost equal to the amount of protein carbonyl groups (30 mol/mol protein) detected. These data suggested that protein modification by acrolein is largely ascribed to the formation of acrolein-lysine and/or acrolein-histidine adducts that possess a carbonyl function. In relation to this, upon incubation of N^{α}-acetyllysine and N^{α}-acetylhistidine with acrolein, we have identified N^{α}-acetyl-FDP-lysine and N^{α}-acetyl-N^{im}-propanalhistidine (Fig. 2), respectively, and found that these adducts indeed are reactive to the carbonyl reagent.

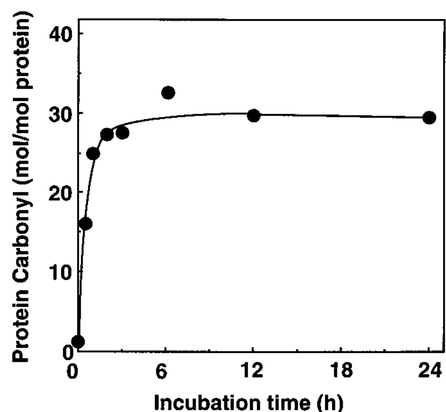

FIG. 1. Introduction of carbonyl groups into protein by reaction with acrolein. BSA (1 mg/ml) was incubated with 1 mM acrolein in 50 mM sodium phosphate buffer (pH 7.4) at 37°C. The protein carbonyl content was determined by the procedure using 2,4-dinitrophenylhydrazine.

mAb Directed to the Acrolein-Modified Proteins. To further verify the presence of acrolein-derived adducts in oxidatively modified proteins, a mAb was raised against the acrolein-modified KLH. During the preparation of the mAbs, hybridomas were selected by comparing the reactivities of the culture supernatant to acrolein-modified BSA, native LDL, and oxidized LDL. Among four clones obtained, the clone 5F6 showed the most distinctive recognition of both acrolein-modified BSA and oxidized LDL against native LDL.

Lipid peroxidation yields lipid hydroperoxides as the major initial reaction products whose decomposition generates a number of breakdown products such as aldehydes. It is therefore conceivable that the antibody recognizes epitopes originating from other lipid peroxidation products. Hence, we examined the immunoreactivity of mAb5F6 to aldehyde-treated proteins by a direct ELISA. As shown in Fig. 3*A*, among the aldehydes tested, acrolein was the only source of antigenic materials generated in the protein. In addition, binding of the acrolein-modified protein to mAb5F6 was scarcely inhibited by N^{α}-acetyl-N^{im}-propanalhistidine but significantly inhibited by N^{α}-acetyl-FDP-lysine (Fig. 3*B*), indicating that FDP-lysine is an epitope of mAb5F6.

Localization of Protein-Bound Acrolein in Human Atherosclerotic Lesions. Early atherosclerosis is characterized by fatty streaks composed primarily of multiple layers of macrophage-derived foam cells and the diffuse intima thickening. Atherosclerotic tissues were examined immunohistochemically for acrolein adducts using polyclonal antiserum and mAb5F6. Acrolein adducts were identified, by both mAb5F6 (Fig. 4*B*) and polyclonal antiserum (Fig. 4*C*), in the macrophage-derived foam cells positive for CD68 (Fig. 4*A*) and in the thickening neointima of arterial walls. Strong staining at the areas, other than granular cytoplasmic elements of foam cells, may therefore be attributed to acrolein-modification of noncellular components in the surrounding sclerotic stroma. Preadsorption of the antibody by N^{α}-acety-FDP-lysine abolished the immunostaining (Fig. 4*D*), indicating the specific reactivity of the antibody (mAb5F6) with epitope. Nonimmune rabbit or mouse IgG gave no immunostaining pattern. These results indicate that acrolein-modified proteins are formed and accumulated in the atherosclerotic lesions.

Peroxidation of Polyunsaturated Fatty Acids Generates Antigenic Materials in the Presence of Protein. Various lines of evidence indicate that an important part of the pathogenesis of atherosclerosis is the oxidative modification of plasma LDL (12–16). To verify the formation of protein-bound acrolein in

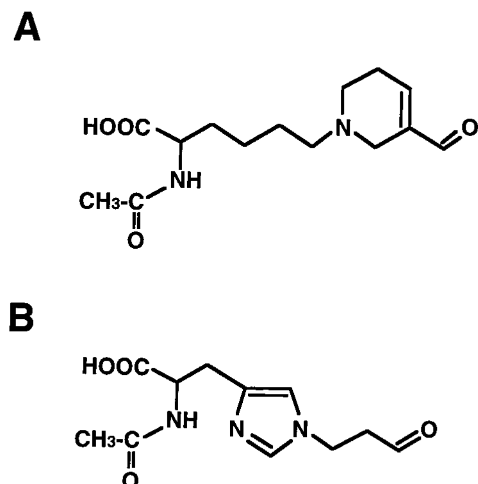

FIG. 2. Structure of N^{α}-acetyl-N^{ε}-(3-formyl-3, 4-dehydropiperidino)lysine (N^{α}-acetyl-FDP-lysine) (*A*) and N^{α}-acetyl-N^{im}-propanalhistidine (*B*).

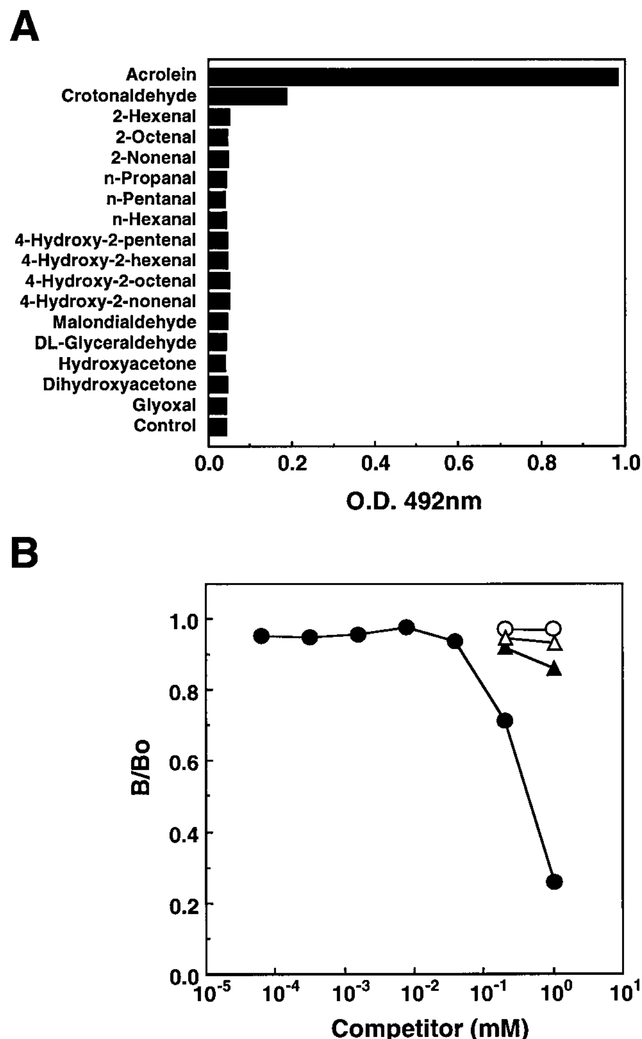

FIG. 3. Specificity of mAb5F6. (*A*) Immunoreactivity of mAb5F6 to the aldehyde-treated protein. Affinity of mAb5F6 was determined by a direct ELISA using aldehyde-treated BSA as the absorbed antigen. A coating antigen was prepared by incubating 1 mg of BSA with 1 mM aldehyde in 1 ml of 50 mM sodium phosphate buffer, pH 7.4, for 2 h at 37°C. (*B*) Competitive ELISA with FDP-lysine. Competitors: ○, N^{α}-acetyllysine; ●, N^{α}-acetyl-FDP-lysine; △, N^{α}-acetylhistidine; ▲, N^{α}-acetyl-N^{im}-propanalhistidine

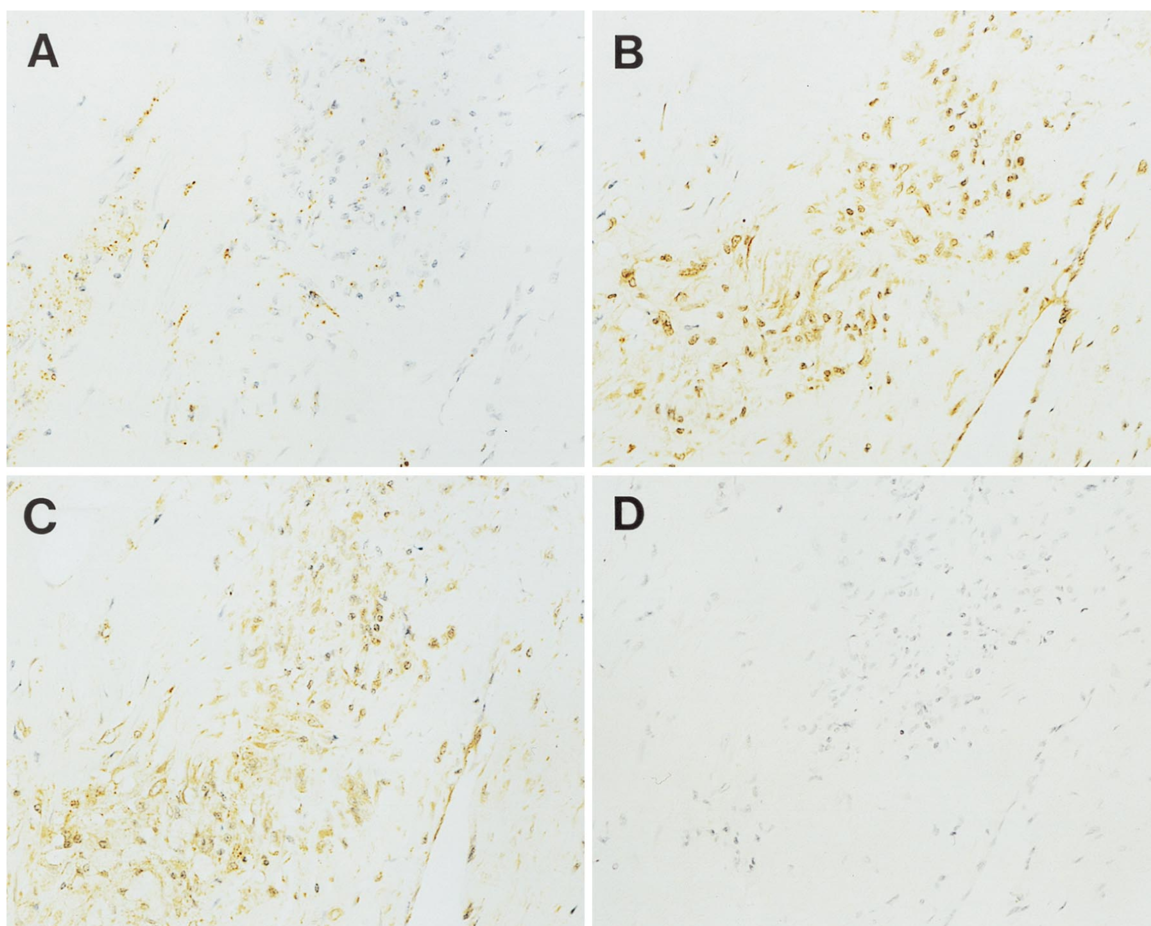

FIG. 4. Immunohistochemical detection of protein-bound acrolein in fatty streak lesions of arterial tissue. Arterial tissue specimen from a 69-year-old male with atherosclerosis was immunostained with anti-CD68 antibody (*A*), mAb5F6 (*B* and *D*), and rabbit polyclonal antiserum raised against acrolein-modified KLH (*C*). For a competitive experiment, mAb5F6 preincubated with an excess of N^{α}-acetyl-N^{ε}-(3-formyl-3, 4-dehydropiperidino)lysine (N^{α}-acetyl-FDP-lysine) was used (*D*). The nuclei were counterstained with Meyer's hematoxylin. The staining with polyclonal antiserum and mAb5F6 was positive in fatty streak lesions. (×200.)

oxidatively modified LDL, the oxidized LDL was analyzed by a direct ELISA using mAb5F6. As shown in Fig. 5*A*, *in vitro* peroxidation of LDL with Cu^{2+} generated antigenic materials. Immunoblot analysis also attested to the formation of protein-bound acrolein in the oxidized LDL (data not shown). These results suggest that acrolein is a lipoperoxidation product that leads to the modification of LDL apolipoprotein B.

To characterize the formation of antigenic materials during lipid peroxidation reactions, model experiments were carried out in which arachidonate was oxidized with an iron/ascorbate-mediated free radical generating system. As shown in Fig. 5*B*, the iron/ascorbate-mediated oxidation of arachidonate in the presence of protein resulted in a time-dependent increase in the antigenecity of protein, whereas the incubation with either iron or ascorbate alone scarcely generated antigenic materials, suggesting that lipid peroxidation is essential for production of protein-bound acrolein. In addition, formation of antigenic materials was also observed in the autoxidation of other polyunsaturated fatty acids, such as linoleate, *cis*-5,8,11,14,17-eicosapentaenoic acid, or *cis*-4,7,10,13,16,19-docosahexaenoic acid in the presence of protein (data not shown). These results suggest that polyunsaturated fatty acids are potential sources of acrolein that causes the production of protein-bound acrolein *in vivo*.

DISCUSSION

In the present study, we have obtained a murine mAb, mAb5F6, that clearly distinguished the acrolein-modified BSA and oxidized LDL from native BSA and LDL. It appears that the antibody is specific to the acrolein-lysine adduct (FDP-lysine) (Fig. 3*B*). Using this antibody, it was clearly shown that atherosclerotic lesions contained antigenic materials in the granular cytoplasmic elements of foam cells and the thickening neointima of arterial walls (Fig. 4). In addition, the presence of antigenic materials has also been observed in nigral neurons of patients with Parkinson disease (N. Hattori, personal communication), in which oxidative stress and mitochondrial respiratory failure with a resultant energy crisis have been implicated as two major contributors to nigral neuronal death (17). It is, therefore, expected that the protein-bound acrolein is potentially one of the most important markers of oxidative stress and that the antibody (mAb5F6) discloses the physiological significance of acrolein in the pathogenesis of numerous diseases associated with oxidative stress.

It is known from the *in vitro* studies that all of the major cell types within the atherosclerotic lesions are capable of promoting the oxidation of LDL (16, 18–23); therefore, the cell-associated staining patterns we observed (Fig. 4) may be attributed to the cellular oxidation of LDL by endothelial cells, macrophages, and/or smooth muscle cells. The resulting oxidized LDL may be taken up by cells and may be the ultimate source of the lipids that accumulate in atherosclerotic lesions. In addition, intracellular granular staining observed in atherosclerotic lesions represents the presence of acrolein adducts that had already been taken up by macrophages and are present within the cell in cytoplasmic organelles. Thus, our results are consistent with the view (12) that protein alter-

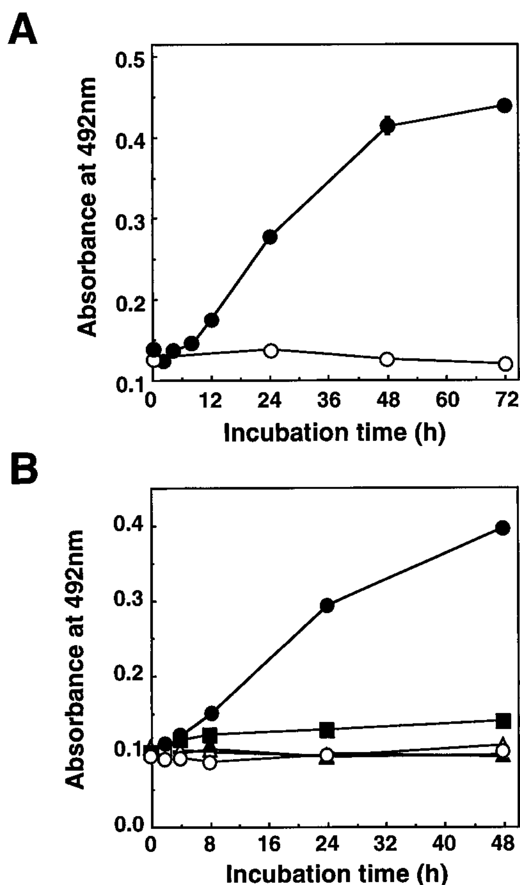

FIG. 5. In vitro formation of protein-bound acrolein via lipid peroxidation. (*A*) Formation of protein-bound acrolein in the oxidized LDL. LDL (0.5 mg/ml) was incubated in the absence (○) or presence of 5 μM Cu^{2+} (●) in 50 mM sodium phosphate buffer (pH 7.4) at 37°C. (*B*) Formation of protein-bound acrolein in BSA during the iron-catalyzed oxidation of arachidonate. The iron-catalyzed oxidation of arachidonate in the presence of BSA was performed by incubating BSA (1 mg/ml) with 2 mM arachidonate in the presence of either 10 μM Fe^{2+}, 1 mM ascorbate, or 10 μM Fe^{2+} and 1 mM ascorbate in 0.1 ml of 0.1 M sodium phosphate buffer (pH 7.4) at 37°C. ○, Control (without arachidonate); ◇, arachidonate; □, Fe^{2+} + arachidonate; ■, ascorbate + arachidonate; ●, Fe^{2+}/ascorbate + arachidonate.

ations, including oxidative modification, predisposes LDL to clearance by scavenger receptors of the macrophages, intracellular deposits of lipoprotein-derived cholesterol, and the formation of foam cells. It is, therefore, suggested that acrolein may play an important role in the formation of arterial foam cells and contribute to the development of atherosclerosis, while additional studies will be needed to establish the direct connection between the acrolein modification of proteins and their atherogenic properties.

It is notable that the exposure of proteins to acrolein introduces carbonyl groups that can be detected using a standard spectrophotometric assay (Fig. 1). Protein carbonyls have represented a putative marker of oxidatively modified proteins, which have been detected in a number of human tissues; in some studies, increased carbonyl levels have been observed in proteins from elderly individuals (24, 25). Because such damage can adversely affect cellular enzymes, the increased protein carbonyls found in Alzheimer disease and amyotrophic lateral sclerosis patients have been causally implicated in these and other degenerative states associated with aging (24, 26, 27). However, although *in vivo* oxidative damage to cell proteins is well established, the cellular pathways involved in the generation of these lesions have not been established. In addition to the large body of data demonstrating the formation of carbonyl groups by metal-catalyzed oxidation systems, our present findings add acrolein to a growing list of lipid peroxidation products that can introduce carbonyl groups into proteins, such as 4-hydroxy-2-nonenal (6), malondialdehyde (28), and lipid peroxy radicals (29).

During the lipid peroxidation process, decomposition of lipid hydroperoxides leads to the generation of many compounds as reactive intermediates. In turn, these can bind to amino acid residues of proteins, generating relatively stable end products. The chemical nature of the products has been elucidated, in part, by detecting the amino acid adducts with highly reactive lipid peroxidation-derived aldehydes, such as 4-hydroxy-2-nonenal (3–6, 11, 30), malondialdehyde (3, 30), and glyoxal (31). It has been suggested that, like 4-hydroxy-2-nonenal, acrolein preferentially reacts with cysteine, histidine, and lysine residues to generate Michael addition-type acrolein-amino acid adducts (3); however, upon the reaction of acrolein with the lysine derivative, we have recently isolated a unique condensed-ring product FDP-lysine. The formation of FDP-lysine may be reasonably explained by the mechanism that one lysine molecule reacts with two acrolein molecules via Michael addition reactions, followed by aldol condensation and dehydration reactions. However, our preliminary experiment has shown that, upon incubation of a lysine derivative with acrolein, FDP-lysine is exclusively formed even in the presence of a large excess of the lysine molecule (e.g., the lysine:acrolein ratio of 100:1). This result raises the possibility that FDP-lysine is formed via an alternative mechanism. Although a detailed mechanism still remains unclear, it may not be unlikely that, because acrolein is easily polymerized, an acrolein dimer having free aldehyde groups reacts with the lysine amino group, generating the formation of a condensed-ring product such as FDP-lysine.

In vitro studies of the detection of protein-bound acrolein during the peroxidation of LDL (Fig. 5*A*) and the metal-catalyzed oxidation of arachidonate in the presence of protein (Fig. 5*B*) demonstrated that substantial amounts of acrolein could be generated during the peroxidation of polyunsaturated fatty acids. In addition to the nonenzymatic oxidation of polyunsaturated fatty acids, the enzymatic oxidation of free hydroxy-amino acids with myeloperoxidase in the presence of H_2O_2 and chloride ion has also been shown to generate acrolein (32). In any case, the observations (33, 34) that (*i*) myeloperoxidase catalyzes lipoprotein oxidation *in vitro*, (*ii*) myeloperoxidase is expressed in human atherosclerotic lesions, and (*iii*) myeloperoxidase can generate reactive species that damage lipids suggest that this heme protein may play a role in the generation of acrolein *in vivo*. Although the mechanism of the formation of acrolein during lipid peroxidation has not yet been experimentally resolved, there may be no doubt that acrolein is a physiologically important aldehyde that could be ubiquitously generated in biological systems. In addition, because acrolein is the most reactive and cytotoxic aldehyde (3), it is also suggested that acrolein is a major causal factor that contributes to the development of tissue damage under oxidative stress.

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