Tandem Mass Spectrometric Characterization of a Specific Cysteic Acid Residue in Oxidized Human Apoprotein B-100

Odile Burlet,* Chao-Yuh Yang, John R. Guyton,† and Simon J. Gaskell‡

Department of Medicine, Baylor College of Medicine, Houston, Texas, USA

The oxidation of low density lipoprotein (LDL) in vivo may result in its unregulated uptake by macrophages, with the consequent accumulation of cholesterol that is characteristic of the development of atherosclerosis. This paper describes initial experiments to elucidate structural changes that occur in an in vitro model of LDL oxidation. LDL was isolated from human blood and oxidized in the presence of copper ion. Lipid was removed and the isolated apoprotein was subjected to tryptic hydrolysis. The hydrolysate was separated by high performance liquid chromatography and individual fractions were screened by amino acid analysis to detect cysteic acid residues. Appropriate fractions were analyzed by fast atom bombardment mass spectrometry and hybrid tandem mass spectrometry. In this manner a tryptic fragment was identified that corresponded to residues 4187–4195 (EELCTMFIR), in which the cysteine and methionine residues were oxidized to cysteic acid and methionine sulfoxide, respectively. Identical analysis of LDL not subjected to in vitro oxidation revealed no evidence for this oxidized peptide. Earlier work established a surface location for this cysteine residue (Cys24) on the LDL particle, which suggested that its modification may significantly affect the properties of LDL, such as the propensity to intermolecular interaction via disulfide bridges. The analytical protocol developed here (involving proteolysis, screening of peptide fragments, and tandem mass spectrometry analysis) constitutes a strategy of general applicability to the characterization of targeted modifications of large proteins via mass spectrometry. (*J Am Soc Mass Spectrom* 1995, 6, 242–247)

Low density lipoprotein (LDL) is a major contributor to the development of atherosclerosis. There is now ample evidence, from both in vitro and in vivo experiments, that oxidative modification of LDL results in significant changes in biological properties [1]. Thus, oxidized LDL shows a reduced rate of cellular uptake via the LDL receptor and an increased rate of uptake via both the scavenger (acetyl-LDL) receptor and a distinct oxidized-LDL receptor found on macrophages [2–5]. These changes in receptor interaction can account for unregulated uptake of oxidized LDL by macrophages and accumulation of free and esterified cholesterol. Structural changes in apolipoprotein B-100 (apo B-100), the principal protein constituent of LDL, are thought to be responsible for the altered receptor-binding properties of oxidized LDL [6]. The

still poorly defined structural modifications of the protein component of LDL that accompany oxidation are undoubtedly complex and may include direct oxidation of the protein as well as the formation of products of interaction of the protein and oxidized lipids. In addition, there is evidence for both protein cleavage [7] and intermolecular cross-linking [8, 9] as a result of oxidation.

The primary sequence of apo B-100 has been established from the complementary DNA sequence [10–14] and substantially confirmed by classical sequencing methods [14, 15]. The locations of cystine bridges, free cysteine residues [16], and sites of glycosylation [15] also have been determined. Hunt et al. [17] have previously reported the tandem mass spectrometry analysis of peptides obtained by a limited trypsin digestion of cyanogen bromide-treated apoprotein B. In the research program of which the present report constitutes a part, the specific concern is with the characterization of structural modifications that accompany oxidation of LDL. Conventional protein analysis techniques are not readily applicable to the characterization of modified proteins; accordingly, tandem mass spectrometry has been used here to analyze tryptic fragments of apo B-100 from oxidized LDL. This initial study has used

Address reprint requests to Simon J. Gaskell, Department of Chemistry, UMIST, Manchester M60 1QD, UK.

* Also at the Department of Chemistry, University of Houston, Houston, TX 77204. Current address: Laboratoire de Pharmacologie et Toxicologie Fundamentales, CNRS, 31077 Toulouse, France.

† Current address: Department of Medicine, Duke University Medical Center, Durham, NC 27710.

‡ Current address: Department of Chemistry, University of Manchester Institute of Science and Technology (UMIST), Manchester M60 1QD, UK.

an in vitro model of the oxidation of LDL. Recognizing the expected complexity of structural changes that accompany LDL oxidation, the initial phase of this study has focused on a specific issue—the recognition and location of sites of oxidation of cysteine residues to cysteic acid. Because it is known that apo B-100 can be cross-linked to apolipoprotein (a) via disulfide bonds [18, 19], the possible modification of cysteine residues is relevant to the alteration of the biological properties of apo B-100 following oxidation.

Experimental

Preparation of Oxidized LDL

LDL was isolated from the whole blood of three healthy human volunteer donors in accordance with standard procedures [20]. Plasma was separated from cells by centrifugation of whole blood at 5000 rev/min for 15 min at 5 °C. By using appropriate densities of sodium chloride and potassium bromide solutions and ultracentrifugation, each lipoprotein component of plasma (chylomicrons, very low density lipoprotein, intermediate density lipoprotein, LDL, and high density lipoprotein) was isolated successively. LDL was thus purified by using a potassium bromide solution (density 1.063) and ultracentrifugation at 55,000 rev/min for 15 h at 5 °C. The layer that contained purified LDL was dialyzed against tris-(hydroxymethyl)amino-methane hydrochloride buffer (pH 7.4) that contained ethylenediaminetetraacetic acid disodium salt for 24 h at 4 °C.

Purified LDL was dialyzed against phosphate buffered saline (PBS; pH 7.4) for 24 h at 4 °C in the dark. The solution (approximately 15 mL of 5.8-mg/mL concentration of apo B-100, as judged by enzyme-linked immunosorbent assay) was diluted to an LDL concentration of about 2 mg/mL with PBS containing 10- μ M copper sulfate. The diluted LDL solution (about 40 mL) was transferred to two dialysis bags (SpectraPor membranes, 12,000–14,000 u molecular weight cutoff; Spectrum Medical Industries, Houston, TX), which were immersed in 40 mL of 10- μ M copper sulfate in PBS. Oxygen was bubbled through the solution for 24 h at 45 °C. [In parallel studies (C.-Y. Yang, unpublished data) it has been established that oxidations at 37 and 45 °C effect a similar extent of total conversion of cysteine to cysteic acid, as judged by amino acid analyses of the total protein.] The LDL solution was then dialyzed against water for 24 h at 4 °C and lyophilized.

Preparation and Tryptic Hydrolysis of Apo B-100

Lyophilized LDL was placed in a dessicator that contained sodium hydroxide pellets and the dessicator was left under vacuum overnight. To each portion of dry LDL was added 5 mL of a 2/1 (v/v) mixture of chloroform and methanol. Each precipitate was sepa-

rated by centrifugation and washed with several portions of the chloroform-methanol solution. The precipitates were dried under a stream of nitrogen and stored under nitrogen at -20 °C.

Delipidated LDL (apo B-100; approximately 43 mg or 83 nmol) was suspended in 3.5 mL of 0.1-M ammonium bicarbonate, pH 7.9. To the suspension was added 1 mg of L-1-tosylamide-2-phenylethylchloromethyl ketone-treated trypsin. The mixture was stirred at 20 °C for 24 h and was centrifuged at 3500 rev/min for 10 min. The supernatant was stored at -20 °C.

The supernatant from the apo B-100 tryptic digest was separated by using a Vydac (Hesperia, CA) reverse-phase C18 high performance liquid chromatography (HPLC) column (4.6 $\times$ 200 mm) heated at 50 °C. The aqueous phase (A) was 0.1% trifluoroacetic acid (TFA) and the organic phase (B) was a 95/5 (v/v) mixture of acetonitrile and water that contained 0.08% TFA. The flow rate was 1.5 mL/min. Detection was by UV absorption at 220 nm. The gradient used to collect fractions for amino acid analyses was from 0% B to 70% B in 70 min and from 70% B to 100% B in the following 5 min. For this analysis a 25- μ L aliquot of tryptic digest was injected (which corresponds to about 0.6 nmol). Fractions were collected and dried under vacuum (Savant Speed-Vac Concentrator, Forma Scientific, Marietta, OH). An equivalent gradient was used for the collection of fractions for mass spectrometry analyses. Aliquots of 200 and 250 μ L of tryptic digest were injected for a total volume of 1.65 mL (which corresponds to a maximum of approximately 39-nmol protein). Fractions were collected, combined, and lyophilized. Each fraction was redissolved in 50 μ L of a 1/1 (v/v) mixture of water and methanol for mass spectrometry analyses. Aliquots of 2 μ L were used for each mass spectrometry analysis (which corresponds to a maximum of approximately 1.5 nmol).

Amino Acid Analysis

HPLC fractions obtained from the apo B-100 digest were placed in a vacuum container. To the container were added 400 μ L of 6-M hydrochloric acid and 20 μ L of 0.02-M phenol. The container was then evacuated for 5 min and heated at 150 °C for 70 min in a preheated oven. The hot container was opened to the atmosphere to remove hydrochloric acid vapors and placed under vacuum for 30 min. To each dry fraction were added 20 μ L of 0.4-N borate buffer (pH 10.4). Samples were vortex mixed, capped, and subjected to automated amino acid analysis. A Hewlett-Packard (Avondale, PA) 1090 HPLC for AminoQuant analysis was used. Aliquots (5 μ L) of hydrolyzed apo B-100 fractions in solution in 0.4-N borate buffer (pH 10.4) were automatically mixed in the injection loop with an additional 5- μ L buffer, 1 μ L of orthophthalaldehyde, and 1 μ L of 9-fluoronylmethylchloroformate. The total volume was then injected onto an octadecylsilyl Hypersil (5- μ m) HPLC column (2.1 $\times$ 200 mm) (Cel Asso-

ciates, Houston, TX) heated at 40 °C. The mobile phase and gradient elution profile were adjusted to optimize chromatographic separation of the cysteic acid derivative. Thus, the eluting solvents consisted of 20-mM sodium acetate, 0.018% (v/v) triethylamine, 10^{-5} -M ethylenediaminetetracetic acid disodium salt, adjusted to pH 6.3 with 1% acetic acid (solvent A), and 0.1-M sodium acetate adjusted to pH 7.2 with 1% acetic acid-methanol-acetonitrile (1/2/2, v/v/v; solvent B). The flow rate was 0.45 mL/min. The solvent program was from 0% B to 60% B in 17 min, 60% B to 100% B in 1 min, isocratic at 100% B for 6 min, and back to 0% B in 1 min. The column was then equilibrated for the next injection with 100% A for 8 min. Detection was by UV absorption with simultaneous and independent monitoring at 238 and 262 nm.

Mass Spectrometry

All analyses were performed using a VG ZAB SEQ instrument (Fisons Instruments/VG Analytical, Manchester, UK) with the configuration BEqQ (magnetic sector-electric sector-rf-only quadrupole-quadrupole mass filter). Ionization was by fast-atom bombardment (FAB), with 8-keV xenon atoms used as the primary beam. The liquid matrix was thioglycerol-2,2'-dithiodiethanol (1/1, v/v), saturated with oxalic acid. Data were recorded via a VG 11-250 data system. Conventional mass spectra were acquired (by scanning of B) in centroided form; individual scans were of approximately 10-s duration. For tandem mass spectrometry analyses, precursor ions were selected at unit resolution by using BE and subjected to collisional activation in the rf-only quadrupole. The collision gas was argon at an estimated pressure of 1.6 to 3.4×10^{-4} mbar (as judged from the recorded manifold pressure and the estimated conductance of the system). These conditions achieved an attenuation of the precursor ion beam of 50-60%. Product ion spectra were recorded by scanning Q, with total scan times of 10-15 s. The mass resolution of product ions was 1.5-2 u. Data were acquired by using the data system "multichannel analyzer" mode, with accumulation of 10-20 scans. In selected cases, tandem mass spectrometry data also were recorded after conversion of the appropriate peptide fraction to the *N*-acetyl derivative. The derivatization procedure was conducted on the FAB sample probe: to the liquid matrix were added 2 μ L of the peptide mixture, dissolved in methanol and water (1/1), and 2 μ L acetic anhydride. The reaction was allowed to proceed for 10 min at 20 °C before the probe was inserted into the mass spectrometer.

Control Experiment

A further sample of LDL (which corresponded to approximately one third of the quantity used in the above procedures) was isolated and treated as described except that the oxidation step was omitted.

Mass spectrometric analyses were performed on selected HPLC fractions.

Results

Figure 1 shows the HPLC profile for the separation (with UV detection) of apo B-100 tryptic peptides from oxidized LDL. The highly complex pattern reflects the multitude of proteolytic cleavage points in a protein of this size ($M_r = 513$ kDa). To screen for cysteic acid-containing fragments, 75 fractions (which correspond to elution time windows of between 0.6 and 1.5 min) were collected and subjected to automated amino acid analysis. The majority of such analyses revealed no evidence of the presence of cysteic acid. Figure 2 shows the amino acid analysis for the fraction collected between 20.8 and 21.4 min. A minor peak was observed at approximately 2.6 min, which corresponds to the elution time of the authentic cysteic acid derivative. [The distortion of the HPLC peak (at 2.7 min) that corresponds to the derivative of aspartic acid was not reproducibly observed. Its origin in this example is not clear.] The low abundance of the putative cysteic acid derivative peak, relative to the peaks that correspond to other amino acids, reflected in part the fact that the HPLC fraction was a complex mixture of tryptic fragments (see succeeding text), only one of which may have contained cysteic acid. Amino acid analysis of one other fraction (with an HPLC retention time of 11.6-12.6 min) revealed the presence of cysteic acid at similar relative concentration. Seven other fractions provided tentative evidence for the presence of cysteic acid, but at a much lower relative concentration.

Figure 3 shows the fast atom bombardment-mass spectrometry (FAB-MS) analysis of the HPLC fraction with a retention time of 20.8-21.4 min; multiple constituents are evident. Tandem mass spectrometry anal-

Figure 1. HPLC separation with UV absorbance detection of the tryptic digest of apo B-100 derived from oxidized LDL. The solvent gradient (%B) is indicated. See Experimental for details.

Figure 2. HPLC separation with UV absorbance detection of amino acid derivatives from the hydrolysis of the tryptic digest fraction with a HPLC retention time of 20.8–21.4 min (Figure 1).

yses of individual constituents provided sufficient information to allow mapping of eight components of this fraction onto the apo B-100 sequence; the results are summarized in Table 1. The underlined sections of each sequence indicate the proportion deduced from the tandem mass spectrometry data. Residues shown in *Italic type* indicate ambiguity with respect to the order of particular residues and/or the differentiation of leucine and isoleucine residues. With two exceptions, each peptide constituent of this fraction corresponded to cleavage at expected sites of tryptic hydrolysis. The component with an $[M + H]^+$ ion of m/z 718 yielded product ions consistent with the sequence LIFDPN and therefore represented cleavage on the C-terminal side of an asparagine residue. There is literature precedent for a nonenzymatic cleavage of this type: it has been suggested that cleavage proceeds via initial succinimide formation with subsequent hy-

drolysis to yield a C-terminal asparagine [21]. The component with an $[M + H]^+$ ion of m/z 777 and attributed to the sequence SFSIPVK follows a tyrosine residue in the apo B-100 sequence and therefore appears to represent a product of combined chymotryptic and tryptic cleavage. It is likely that the appearance of

Table 1. Peptides identified in a HPLC fraction (20.8–21.4-min retention time) of the tryptic digest of apo B-100 from oxidized LDL

$[M + H]^+^a$	Peptide sequence ^b	Residue numbers in apo B-100 sequence
621	<u>LT</u> /FK	4009–4013
718 ^c	<u>L</u> /FDPN ^d	632–637
777 ^c	SFS/ <u>PVK</u> ^e	3691–3697
1021	<u>NNA</u> LDFVTK	3186–3194
1058	<u>LS</u> /LTDPDGK ^d	4425–4434
1205	<u>EELC</u> *TM ^f F/R	4187–4195
1242	ATGVLYDYVNK	4050–4060
1406	LQTFLDDASPGDK ^g	518–530

^a Mass-to-charge ratio of the protonated molecule, rounded down to the nearest integral value.

^b Conventional single letter codes are used, except for: C*, cysteic acid; M^o, methionine sulfoxide. Underlined portions of the sequence were deduced from the tandem mass spectrometry analysis alone. Amino acid codes in *italic type* represent ambiguities of interpretation of the mass spectrometry data with respect to leucine and isoleucine isomers or the ordering of a pair of residues.

^c Sequence determined from tandem mass spectrometry of the underivatized peptide and of the *N*-acetyl derivative.

^d Sequence follows an asparagine residue (according to the expected apo B-100 sequence), which indicates nonenzymatic cleavage.

^e Sequence follows a tyrosine residue (according to the expected apo B-100 sequence), which indicates chymotryptic cleavage.

^f Sequence follows a leucine residue (according to the expected apo B-100 sequence), which indicates chymotryptic cleavage.

Figure 3. FAB mass spectrum of the tryptic digest fraction with a HPLC retention time of 20.8–21.4 min (Figure 1).

products other than those predicted as a result of tryptic hydrolysis alone was promoted by the prolonged hydrolysis time required to achieve efficient tryptic cleavage.

The peptide constituent that yielded an $[M + H]^+$ ion of m/z 1205 (Table 1) was of particular interest in view of the primary focus of this study on cysteic acid-containing components. Figure 4 shows the product ion spectrum obtained by tandem mass spectrometry with low energy collision-activated dissociation (CAD). The C-terminal arginine residue was apparent readily from the Y_1'' ion of m/z 175; all other product ions of significant abundance also were attributable to the Y_n'' series and were consistent with the sequence EELCTMFIR with oxidation of the cysteine and methionine residues to cysteic acid and methionine sulfoxide, respectively. This sequence corresponds to residues 4187–4195 of apo B-100.

The extraordinary clarity of the product ion spectrum obtained for this example has prompted a detailed study of the fragmentation properties of synthetic peptides of related structure. Detailed findings are reported elsewhere [22]. In brief, the formation of multiple members of the Y_n'' series of ions appears to be promoted by a gas-phase interaction between the arginine and cysteic acid residues, which reduces the propensity for protonation exclusively at the C-terminal arginine. The resultant population of protonated precursor ions is heterogeneous with respect to the site of charge, with consequent promotion of cleavage of each peptide bond. The energetics of bond cleavage and the relative basicities of the separating fragments favor charge retention on C-terminal products.

Tryptic hydrolysis and preparative HPLC separation also were performed on apo B-100 from LDL not subjected to oxidation in vitro. The HPLC profile, obtained by UV absorbance detection, was substantially different from that observed for the oxidized protein, which attests to the complexity of the structural changes that accompany oxidation. A fraction

with a retention time of 20.8–21.4 min was collected and analyzed by FAB-MS. The spectrum included ions (m/z 621, 1021, 1242) previously observed in the equivalent fraction derived from the oxidized protein. There was no evidence for a component that yielded m/z 1205. FAB-MS analyses of preceding and following HPLC fractions similarly failed to reveal this component.

Detailed mass spectrometry and tandem mass spectrometry analyses were performed on a further fraction (with an HPLC retention time of 11.6–12.6 min) of the tryptic digest derived from oxidized LDL. As previously noted, amino acid analysis provided evidence that this fraction also included a cysteic acid-containing component. Eight components were identified (including one trypsin autolysis product); none, however, was identified as a cysteine- or cysteic acid-containing peptide.

Discussion

The work described here sought to detect and locate cysteic acid residues in apoprotein B-100 of oxidized LDL. The strategy involved screening by amino acid analysis of HPLC fractions derived from the tryptic digest of the oxidized protein. FAB-MS and hybrid tandem mass spectrometry were used to analyze fractions of interest. The particular value of tandem mass spectrometry in the present application derives from the facility for the determination of sequence without the prior isolation of individual peptide constituents and from the ability to recognize modified peptide structures. Hybrid tandem instruments have not been used as widely to analyze peptides derived from biological sources (as opposed to authentic standards) as their widespread availability might suggest. For the present work, it is noteworthy that approximately unit mass resolution of precursor ions was used and that the resolution of product ions (1.5–2 u) was sufficient to differentiate readily between product ions of adjacent integral mass. In certain instances, essentially complete sequence information was obtained. For most examples, sufficient partial sequences could be deduced to permit mapping of the peptide onto the known apo B-100 sequence. The fragmentation properties of protonated peptides following low energy collision activation remain a subject of investigation [23]. It appears, however, that typical low energy fragmentations are charge-site directed. It is well accepted that the differentiation of the isomeric leucine and isoleucine residues cannot be achieved on the basis of low energy fragmentations [24]. Such distinction can be made from cleavages of the amino acid residue side chains, fragmentation processes that have a high energy requirement [25]. High energy (kiloelectronvolt in the laboratory frame-of-reference) CAD of peptides can be performed by using hybrid instruments, albeit with significant sacrifice in performance by comparison with four sector instruments [26]. Such analyses were not,

Figure 4. Product ion spectrum obtained by CAD of precursor ions of m/z 1205 during the tandem mass spectrometry analysis of the tryptic digest fraction with a HPLC retention time of 20.8–21.4 min (Figure 1).

however, required in the work reported here, which involves the recognition of known or modified tryptic peptides.

The principal finding from this work was the identification of cysteic acid at residue 4190 in apo B-100 of oxidized LDL. Earlier work with native LDL [15] established that the tryptic peptide (EELCTMFIR) that incorporates this residue (Cys24) was partially released by tryptic hydrolysis prior to delipidation of LDL; this sequence resides, however, in a protein domain largely intractable to tryptic hydrolysis without delipidation. Coleman et al. [27] used a fluorescent probe to identify Cys22 and Cys24 as free cysteine residues exposed on the LDL surface and therefore potentially available for intermolecular coupling, for example, to apolipoprotein (a) via a disulfide bond [18, 19]. Carboxymethylation of LDL and subsequent analysis of hydrolysis products has, however, suggested a greater exposure for the Cys22 residue. It is clear that the oxidation of Cys24 (and of the proximal methionine residue) observed in the present work resulted from treatment of the LDL solution with oxygen in the presence of copper ions, rather than occurring as an artifact of the isolation and hydrolysis procedure. Application of an identical work-up procedure to a preparation of LDL not subject to intentional oxidation revealed no evidence of the tryptic peptide that incorporated oxidized Cys24. It has yet to be determined, of course, whether the same structural modification accompanies oxidative modification of LDL in vivo. It has been established, however, that LDL oxidized in the presence of copper ions possesses the same properties (with respect to receptor uptake, cytotoxicity, etc.) demonstrated by LDL oxidatively modified by incubation with endothelial cells [5, 28].

The work described here represents a first stage in an extensive program, the ultimate objective of which is to define the structural modifications that accompany the oxidation of LDL in vivo. The reported determination of the occurrence and location of cysteine oxidation under in vitro conditions may have physiological implications (by extrapolation to the in vivo situation), but may be more significant in exemplifying a suitable general approach to the characterization of specific protein modifications. Thus, the approach involves proteolytic cleavage, screening for the structural modification of interest, and tandem mass spectrometry analyses. This analytical strategy is currently being applied to the determination of modifications of LDL that involve lipid-protein interactions. Beyond studies of LDL, these analyses provide a useful paradigm for the targeted mass spectrometric analysis of specific modifications of large proteins.

Acknowledgments

This work was supported by grants from the National Institutes of Health (GM 48267 to SJG; HL 27341 (Core D) to CYY; HL 45619 to JRG).

References

- Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. *New Engl. J. Med.* **1989**, 320, 915-924.
- Henriksen, T.; Mahoney, E. M.; Steinberg, D. *Proc. Natl. Acad. Sci. USA* **1981**, 78, 6499-6503.
- Henriksen, T.; Mahoney, E. M.; Steinberg, D. *Arteriosclerosis* **1983**, 3, 149-159.
- Sparrow, C. P.; Parthasarathy, S.; Steinberg, D. *J. Biol. Chem.* **1989**, 264, 2599-2604.
- Steinbrecher, U. P.; Witztum, J. L.; Parthasarathy, S.; Steinberg, D. *Arteriosclerosis* **1987**, 7, 135-143.
- Parthasarathy, S.; Fong, L. G.; Otero, D.; Steinberg, D. *Proc. Natl. Acad. Sci. USA* **1987**, 84, 537-540.
- Schuh, J.; Fairclough, G. F.; Haschemeyer, R. H. *Proc. Natl. Acad. Sci. USA* **1978**, 75, 3173-3177.
- Jurgens, G.; Lang, J.; Esterbauer, H. *Biochim. Biophys. Acta* **1986**, 875, 103-114.
- Hoff, H. F.; Whitaker, T. E.; O'Neil, J. *J. Biol. Chem.* **1992**, 267, 602-609.
- Cladaras, C.; Hadzopoulou-Cladaras, M.; Nolte, R. T.; Atkinson, D.; Zannis, V. I. *EMBO J.* **1986**, 5, 3495-3507.
- Chen, S.-H.; Yang, C.-Y.; Chen, P.-F.; Setzer, D.; Tanimura, M.; Li, W.-H.; Gotto, A. M.; Chan, L. *J. Biol. Chem.* **1986**, 261, 12918-12921.
- Knott, T. J.; Pease, R. J.; Powell, L. M.; Wallis, S. C.; Rall, S. C.; Innerarity, T. L.; Blackhart, B.; Taylor, W. H.; Marcel, Y. L.; Milne, R. W.; Johnson, D.; Fuller, M.; Lusis, A. J.; McCarthy, B. J.; Mahley, R. W.; Levy-Wilson, B.; Scott, J. L. *Nature* **1986**, 323, 734-738.
- Law, S. W.; Grant, S. M.; Higuchi, K.; Hospattankar, A.; Lackner, K.; Lee, N.; Brewer, H. B. *Proc. Natl. Acad. Sci. USA* **1986**, 83, 8142-8146.
- Yang, C.-Y.; Chen, S.-H.; Gianturco, S. H.; Bradley, W. A.; Sparrow, J. T.; Tanimura, M.; Li, W.-H.; Sparrow, D. A.; DeLoof, H.; Rosseneu, M.; Lee, F.-S.; Gu, Z.-W.; Gotto, A. M.; Chan, L. *Nature* **1986**, 323, 738-742.
- Yang, C.-Y.; Gu, Z.-W.; Weng, S.-A.; Kim, T. W.; Chen, S.-H.; Pownall, H. J.; Sharp, P. M.; Liu, S.-W.; Li, W.-H.; Gotto, A. M.; Chan, L. *Arteriosclerosis* **1989**, 9, 96-108.
- Yang, C.-Y.; Kim, T. W.; Weng, S.-A.; Lee, B.; Yang, M.; Gotto, A. M. *Proc. Natl. Acad. Sci. USA* **1990**, 87, 5523-5527.
- Hunt, D. F.; Yates, J. R., III; Shabanowitz, J.; Winston, S.; Hauer, C. R. *Proc. Natl. Acad. Sci. USA* **1986**, 83, 6233-6237.
- Utermann, G.; Weber, W. *FEBS Lett.* **1983**, 154, 357-361.
- Gaubatz, J. W.; Heideman, C.; Gotto, A. M.; Morrisett, J. D.; Dahlen, G. H. *J. Biol. Chem.* **1983**, 258, 4582-4589.
- Havel, R. J.; Eder, H. A.; Bragdon, J. H. *J. Clin. Invest.* **1955**, 43, 1345-1353.
- Geiger, T.; Clarke, S. *J. Biol. Chem.* **1987**, 262, 785-794.
- Burlet, O.; Yang, C.-Y.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1992**, 3, 337-344.
- Burlet, O.; Orkiszewski, R. S.; Ballard, K. D.; Gaskell, S. J. *Rapid Commun. Mass Spectrom.* **1992**, 6, 658-662.
- Hunt, D. F.; Shabanowitz, J.; Yates, J. R.; Griffin, P. R.; Zhu, N. Z. In *Mass Spectrometry of Biological Materials*; McEwen, C. N.; Larsen, B. S., Eds.; Marcel Dekker: New York, 1990; p 169.
- Johnson, R. S.; Martin, S. A.; Biemann, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, 86, 137-154.
- Burlet, O.; Orkiszewski, R. S.; Gaskell, S. J. *J. Am. Soc. Mass Spectrom.* **1993**, 4, 470-476.
- Coleman, R. D.; Kim, T. W.; Gotto, A. M.; Yang, C.-Y. *Biochim. Biophys. Acta* **1990**, 1037, 129-132.
- Steinbrecher, U. P.; Parthasarathy, S.; Leake, D. S.; Witztum, J. L.; Steinberg, D. *Proc. Natl. Acad. Sci. USA* **1984**, 81, 3883-3887.