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International Journal of Mass Spectrometry 219 (2002) 671–680

International Journal of Mass Spectrometry

www.elsevier.com/locate/ijms

MALDI- and ESI-MS of the HDL apolipoproteins; new isoforms of apoA-I, II

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Received 22 February 2002; accepted 15 April 2002

Abstract

Apolipoproteins isolated from human high density lipoprotein were analyzed by matrix-assisted laser desorption (MALDI) and electrospray ionization mass spectrometry (ESI-MS). The long-term objective of this study is to identify and characterize new apolipoproteins and isoforms for clinical studies incorporating some of the latest advances in analytical chemistry methodology including mass spectrometry. The focus of this paper is on developing an understanding of the link between MALDI and ESI-MS data in the analysis of this complex mixture of proteins. Forty-nine peaks were observed in the MALDI spectrum and 11 species in the ESI-MS spectrum. The MALDI spectra consisted of peaks corresponding to known apoA and apoC molecular ion adducts and 11 species that could not be identified. The ESI-MS data provided excellent confirmation of MW values for the prominent ions in the spectrum. New isoforms of apoA-I and apoA-II were observed corresponding to truncation of C-terminal neutral amino acids. A new kind of isoform of apoA-II was discovered where the double strand is cleaved in vivo and the single strand is cysteinylated at the cleavage site. Confirmation was obtained by studying the change in the MALDI spectrum after performic acid oxidation. Charge-state distributions in the ESI-MS spectra were used to confirm the identity of this new isoform. The charge state distribution in ESI-MS was found to correlate with the number of basic residues in the apolipoprotein. (Int J Mass Spectrom 219 (2002) 671–680)

Keywords: MALDI; ESI; ApoA-I; ApoA-II; New isoforms

1. Some comments about Yannick Hoppilliard

We would like to express our appreciation for Yannick's contributions to PDMS. While physics groups focussing on the mechanism of molecular ion ejection have carried out most of the fundamental studies in PDMS, Yannick was essentially the sole voice concerned about the chemistry of the process. What is controlling molecular ion formation? Are the fragment ions formed at the high excitation energies of PDMS following the same ion dissociation pathways observed in collisionaly-induced fragmentation? As a result of Yannick's contributions, PDMS has a solid theoretical base both from a physics and chemistry perspective.

Our work for the past several years has carried us far from the PDMS field [1] into the medical research realm, applying modern methods of chemical analysis to develop better markers for the detection and treatment of cardiovascular disease. Mass spectrometry is one of the methods employed to characterize the

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1387-3806/02/$ – see front matter
PII S1387-3806(02)00709-1

protein domains of lipoproteins by MALDI [2] and ESI [3]. Mixtures of well-characterized proteins isolated from blood samples and in well-defined concentration ranges are involved in the analysis. In the first MALDI and ESI-MS analyses of these mixtures, it was immediately apparent that the relative intensities of the molecular ions were markedly different comparing the two methods and that gas phase chemistry was playing an important role. In trying to understand these processes, we recalled Yannick's work and the chemical principles she developed. We felt it was appropriate to report these findings in this special issue in honor of Yannick Hoppilliard.

2. Introduction

High density lipoprotein (HDL) plays a major role in lipid metabolism and cholesterol transport. It has a micelle-like spherical structure with a diameter of 10 nm and a MW of 360,000. It consists of a lipid core made up of cholesterol esters (82%) and triglycerides (18%) surrounded by a surface structure consisting of phospholipids (75%), cholesterol (23%), and apolipoproteins (3.5%). The major apolipoprotein, apoA-I (MW 28,078), stabilizes the structure of the HDL particle and is an enzyme activator for lecithin-cholesterol acyltransferase. Its serum concentration is of the order of 5×10^{-5} M. The second most abundant apolipoprotein, apoA-II, is a covalently-linked dimer having a MW of 17,414 and a molar concentration of 2×10^{-5} M. Other minor apolipoproteins include apoC-I, MW 6630, [9×10^{-6} M], apoC-II, MW 8914.9 [1×10^{-6} M], and apoC-III, MW 8764.7, [8×10^{-6} M]. What determines the relative intensities of the molecular ions of these species in a mass spectrum is not only their concentrations but probably their p*I* values and number of basic residues. ApoC-I is the most basic of these proteins (p*I* 6.5), while the p*I* values of the other apos range from 4.9 (apoA-II) to 5.6 (apoA-I). The data for these apolipoproteins were taken from a volume of Methods in Enzymology devoted to the properties and structure of lipoproteins [4].

In previous studies, we reported on the analysis of the apolipoproteins of very-low density lipoprotein (VLDL) by MALDI and ESI-MS. In this study, we detected fifteen isoforms of the apoCs due to post-translational processes. One of these, truncation of N-terminal amino acids, had not been previously observed [5]. The same general techniques used in this study have been applied to the present study. The objective of the study reported here is to search for new isoforms of the major apolipoproteins of HDL (apoA-I and apoA-II) using MALDI and ESI-MS as complementary analyses and to determine which peaks in the spectra are due to artifacts introduced by the mass spectrometry as opposed to actual species in the sample. The HDL apos present a unique situation where the major components of the protein mixture are well characterized and the molar concentrations are known. Post-translational products abound and which ions and their intensities are generated using the same sample for MALDI and ESI-MS analysis may give insights into the still mysterious mechanisms of ionization that occur in the MALDI plume and the shrinking, fissioning charged droplet of the electrospray.

3. Experimental

3.1. Chemical reagents

Chemicals used were: HPLC grade acetonitrile (ACN; EM Science, Gibbstown, NJ), dimethyl sulfoxide and HPLC grade water (Burdick and Jackson Laboratories Inc., Muskegon, MI), formic acid (88% solution, EM Science), sinapinic acid (Aldrich Chemical Co., Milwaukee, WI), sodium azide (Aldrich), sudan black B (SBB) (Sigma), sucrose and 30% w/w hydrogen peroxide (Fisher Scientific, Pittsburgh, PA), trifluoroacetic acid (TFA) (Pierce Chemical Co., Rockford, IL).

3.2. Serum/plasma collection

The HDL apos used in this study were obtained from the serum of a population of healthy, young

college students and from an on-going clinical study involving patients with recent myocardial infarctions. Serum samples were collected by a blood draw into an untreated 7 mL Vacutainer™ tube (Becton Dickinson Systems, Franklin Lakes, NJ). The blood was allowed to clot for 45 min at room temperature followed by a 2000 rpm centrifugation for 20 min at 4 °C. The serum was recovered as the supernatant.

3.3. Separation of lipoprotein fractions by ultracentrifugation (UC)

The collected serum samples were separated into lipoprotein fractions by a single spin density gradient UC method described elsewhere [6]. Briefly, a 20% (w/v) sucrose solution containing sodium azide was used as the density gradient medium. An 800 μL aliquot of this solution was pipetted into a 11 mm × 34 mm polycarbonate open-top centrifuge tube (Beckman Instruments, Fullerton, CA). A 200 μL volume of serum was stained with 7 μL of SBB in DMSO (1% w/v) for visualization, diluted with deionized water to a volume of 400 μL and applied to the top of the sucrose solution. The tube was then centrifuged in a Beckman TL-100 ultracentrifuge using a 30° fixed angle TLA 100.2 rotor at 436,000 × *g* and 20 °C for 6 h. After centrifugation, a 200 μL volume of the HDL fraction ($1.06 < d > 1.10$) was collected by aspiration.

3.4. Delipidation of HDL and recovery of apolipoproteins

The recovery of the apolipoproteins from HDL was carried out using a procedure developed in our laboratory [7]. Briefly, a sample was prepared for delipidation using a tC_{18} Light™ cartridge (Sep-Pack, No. 51910, Waters, Milford, MA) by mixing 100 μL of the HDL fraction with 40 μL of 2.5% (v/v) formic acid and 260 μL water. The cartridge was conditioned with 5 mL of 2.5% (v/v) formic acid in acetonitrile followed by 5 mL of 2.5% (v/v) formic acid in water. After the sample was loaded onto the cartridge, the cartridge was rinsed with 5 mL of 0.1% (v/v) TFA in water to remove any water soluble impurities including the sucrose density gradient solute. Proteins were then eluted in 50 μL aliquots of 2.5% formic acid in acetonitrile. Each 50 μL eluant was tested for protein concentration using the BCA method [8]. The second and third fraction tested positive for protein. The proteins in these two fractions were characterized by MALDI mass spectrometry. The second fraction contained predominantly human serum albumin while the third fraction contained the apolipoprotein fraction. Subsequent MALDI and ESI studies were carried out using samples from the third fraction.

3.5. Performic acid oxidation

A delipidated HDL fraction eluted from the tC_{18} cartridge was treated with performic acid using the following procedure. Performic acid was prepared by mixing 1 mmol of hydrogen peroxide with 40 mmol of formic acid. This solution was then added to a 1 mg/mL solution of delipidated HDL at a 1:1 molar ratio of hydrogen peroxide to apolipoproteins. After a 40-min incubation at room temperature, the oxidized apos were analyzed by MALDI.

3.6. MALDI and ESI analysis

The MALDI analysis was performed using a commercial MALDI-TOF mass spectrometer (Voyager Elite XL, PerSeptive Biosystems, Framingham, MA), [9] in the linear mode. The sample preparation using sinapinic acid as the matrix followed a published procedure [10]. A 1.5 μL volume of the delipidated apolipoprotein mixture (1 mg/mL) was diluted with 6 μL of deionized water and 3 μL of matrix solution (30 mg/mL). A 0.5 μL aliquot of this solution was placed on a thin layer of crystalline matrix on a stainless steel sample plate, dried, and rinsed with water and inserted into the mass spectrometer.

An external mass calibration was used in the developmental phase (bovine insulin, MW 5733.6 and horse heart myoglobin, 16,950.5). For the final measurements, two naturally-occurring components of

the apolipoprotein mixture, apoC-I (MW 6630.6) and apoC-III_1(MW 9421.3) were used as internal standards.

The ESI-TOF instrument was built in house [11]. It has an orthogonal acceleration geometry and a resolution (FWHM) of 1850. The delipidated apolipoprotein mixture from the cartridge was directly injected into the mass spectrometer with a flow rate of 1 μL/min. Spectra were acquired for 10 min. The multiply-charged ions of apoC-I were used as internal mass standards.

4. Results and discussion

4.1. MALDI spectrum of HDL apos

Fig. 1 is the MALDI mass spectrum of the HDL apos. Fig. 1a covers the *m*/*z* range from 6,400 to 12,000. Starting at the low end of the mass spectrum, ApoC-I and its recently reported truncated isoform [5] were observed at *m*/*z* 6630.6 and 6432.5, respectively. The peaks labelled *p*' and *q*' are +98 and 206 Da adducts of apoC-I'. The same adducts, (*p* and *q*) are observed for apoC-I as well. ApoC-II appears at *m*/*z* 8204.2. A small peak 16 Da higher, labelled "Ox", has variable intensity in the serum population. We believe that this apo is very sensitive to in vivo and in vitro oxidation and is a potential marker for oxidation status in lipoproteins. A peak labelled "*l*" at *m*/*z* 8528.9 is a low-intensity peak but appears frequently in the serum population. The origin of this peak is unknown. A triplet of three peaks at *m*/*z* 8689, 8625, and 8563 are from doubly-charged double-stranded apoA-II and two new isoforms discovered in this study. Several low-intensity peaks were observed in the *m*/*z* range between 8500 and 9600 that were consistently observed in serum samples from many donors that could not be identified with any known apos or their adducts. We believe that the proteins associated with these ions are components of the HDL particle. Their *m*/*z* values and labels in Fig. 1a are: 8529 (*l*), 8579 (*m*), 8738 (*n*), 8866 (*x*), 9136 (*t*), 9359 (*y*), 9392 (*z*). Two high-intensity peaks were observed at *m*/*z* 8681.4 and 8809.9 and are labelled as A-II monomer' and A-II monomer, respectively. No previously reported apos have these MW values. Additional studies were carried out to identify the nature of these proteins and the results are discussed below. Continuing up the scale in this mass range, a strong peak is observed at *m*/*z* 8914.4 which corresponds to the MW of proapoC-II. A satellite peak 16 Da higher is due to some proapoC-II that has been oxidized. In the *m*/*z* range from 9,100 to 10,000, the series of apoC-III isoforms gives several peaks that have been previously identified [5].

In the *m*/*z* region from 12,000 to 30,000 (Fig. 1b), a quartet of low-intensity peaks of unknown origin (labelled *k*) but present in all serum samples has been detected at *m*/*z* 12,571, 12,692, 12,860, and 12,961. The region around *m*/*z* 14,000 is associated with the doubly-charged apoA-I ion. A triplet of ions associated with apo-II is centered around *m*/*z* 17,000 and another set of ions around *m*/*z* 28,000 is associated with the major HDL apo, apoA-I. The patterns for apoA-II and apoA-I indicate the presence of isoforms that had not been previously identified. Peaks were observed at *m*/*z* 28,176 and 28,274 which we believe are 98 Da adducts of apoA-I. The 98 Da adducts were observed for apoC-I and apoA-I and are most likely due to addition of phosphoric acid molecules. A second class of adducts due to addition of sinapinic acid matrix with loss of water was also observed for apoC-I, II, III. The identification of these peaks and measured MW values compared to values calculated from their known sequence are given in Table 1.

4.2. ESI spectrum of HDL apos

Fig. 2 shows the ESI spectrum of the same sample used for the MALDI analysis. In contrast to the MALDI spectrum, where over 30 different apo isoforms were identified, only 11 apos were identified in the ESI spectrum. The MW values for these apos derived from the ESI-MS data are included in Table 2 for comparison. A complex pattern of multiply-charged peaks was observed in the *m*/*z* region 700–1600. The apoA-I pattern (labelled "*a*") was readily identified.

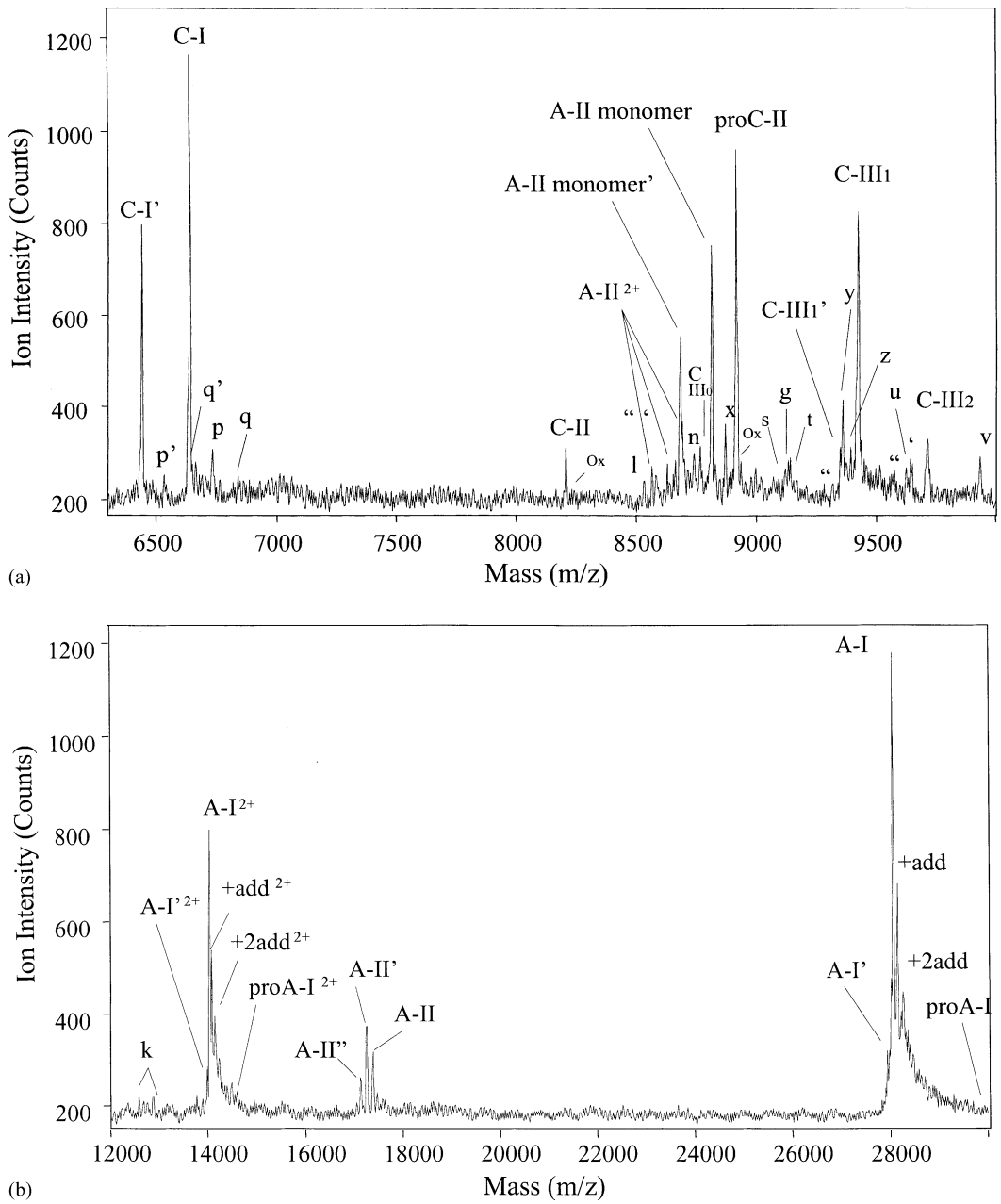

Fig. 1. Positive ion MALDI spectrum of human serum apolipoproteins from delipidated HDL fraction: low-mass (*m*/*z*) region (a) and high-mass region (b).

The most abundant charge state was at 32+ and the distribution extended to 42+. The second most intense distribution is due to the double-stranded apoA-II labelled "*B*". This distribution peaks at 14+ and extends to +18. Several smaller intensity patterns were observed and could be identified by a correlation of what peaks were observed in the MALDI spectrum.

Table 1
Calculated and experimental MW values of the human serum HDL apolipoproteins identified in this study (Figs. 1 and 2)[a]

Code	Identification	MW_{CALC}	MW_{exp} ± S.D., MALDI	MW_{exp} ± S.D., ESI
C-I'	ApoC-I minus N-terminus Thr-Pro-	6432.4	6432.5 ± 0.1	6432.2 ± 0.2
p',	ApoC-I' + 98 Da adduct	6530.5	6530.3 ± 0.8	
C-I	ApoC-I	6630.6	6630.6 calibr.	6630.6 calibr.
Q',	ApoC-I' + 206 Da adduct	6638.6	6638.8 ± 2.0	
p,	ApoC-I + 98 Da adduct	6728.7	6728.0 ± 0.8	
q,	ApoC-I + 206 Da adduct	6836.8	6837.1 ± 0.8	
C-II	ApoC-II	8204.1	8204.2 ± 0.6	
$C\text{-}II_{ox}$	Oxidized apoC-II, apoC-II + 16 Da	8220.1	8219.6 ± 2.0	
l	Unidentified		8528.9 ± 1.5	
$A\text{-}II''^{2+}$	ApoA-II minus two C-terminus -Gln	17125.6	17124.3 ± 2.0	
m	Unidentified		8579.0 ± 2.0	
$A\text{-}II'^{2+}$	ApoA-II minus C-terminus -Gln	17253.7	17250.3 ± 3.0	
$A\text{-}II_{monomer}$'	Single chain apoA-II minus C-terminus -Gln	8581.8	8681.4 ± 0.5	
$A\text{-}II^{2+}$	ApoA-II	17379.8	17378.7 ± 1.0	
n	Unidentified		8738.5 ± 1.0	
$C\text{-}III_0$	$ApoC\text{-}III_0$	8765.7	8764.3 ± 0.9	
$A\text{-}II_{monomer}$	Single chain apoA-II	8809.9	8809.9 ± 0.5	8809.3 ± 0.6
x	Unidentified		8865.7 ± 1.5	
ProC-II	ProapoC-II	8914.9	8914.4 ± 0.7	8914.4 ± 0.7
$ProC\text{-}II_{ox}$	Oxidized proapoC-II, proapoC-II + 16 Da	8930.9	8930.4 ± 1.5	
s,	PropapoC-II + 206 Da adduct	9121.1	9120.8 ± 2.0	
$C\text{-}III_{0,Glyc}$'	Galβ1, 3GalNAc-O-$apoC\text{-}III_0$	9130.0	9130.2 ± 2.0	
t	Unidentified		9135.9 ± 2.3	
$C\text{-}III_1$"	$ApoC\text{-}III_1$ minus C-terminus -Ala-Ala	9279.1	9278.4 ± 2.0	
$C\text{-}III_1$'	$ApoC\text{-}III_1$ minus C-terminus -Ala	9350.2	9348.6 ± 1.5	
y	Unidentified		9358.9 ± 0.6	
z	Unidentified		9392.1 ± 1.0	
$C\text{-}III_1$	$ApoC\text{-}III_1$	9421.3	9421.3 calibr.	9420.3 ± 2.0
$C\text{-}III_2$"	$ApoC\text{-}III_2$ minus C-terminus -Ala-Ala	9570.4	9569.4 ± 3.0	
u	$ApoC\text{-}III_1$ + 206 Da adduct	9627.5	9625.4 ± 2.0	
$C\text{-}III_2$'	$ApoC\text{-}III_2$ minus C-terminus -Ala	9641.5	9640.6 ± 2.0	
$C\text{-}III_2$	$ApoC\text{-}III_2$	9712.6	9713.3 ± 1.7	9712.4 ± 3.0
v	$ApoC\text{-}III_2$ + 221 Da isoform		9933.3 ± 0.9	
*k*1	Unidentified		12571.5 ± 2.0	
*k*2	Unidentified		12692.7 ± 3.0	
*k*3	Unidentified		12680.1 ± 2.0	
*k*4	Unidentified		12961.5 ± 2.0	
$A\text{-}I'^{2+}$	ApoA-I without C-terminus -Gln	27950.5	27949.9 ± 2.0	27955 ± 5
$A\text{-}I^{2+}$	ApoA-I	28078.6	28078.0 ± 1.0	28078.8 ± 1.0
$+add^{2+}$	ApoA-I + 98 Da adduct	28176.7	28176.7 ± 1.5	
$+2add^{2+}$	ApoA-I + two 98 Da adduct	28274.8	28277.3 ± 2.0	
$ProA\text{-}I^{2+}$	ProapoA-I	28961.6	28959.4 ± 3.0	
A-II"	ApoA-II minus two C-terminus -Gln	17125.6	17122.9 ± 1.0	17123.0 ± 1.5
A-II'	ApoA-II minus C-terminus -Gln	17253.7	17249.7 ± 0.8	17250.9 ± 1.0
A-II	ApoA-II	17379.8	17377.9 ± 1.0	17379.7 ± 1.0
A-I'	ApoA-I minus C-terminus -Gln	27950.5	27953 ± 9	
A-I	ApoA-I	28078.6	28075.0 ± 3.0	
+add	ApoA-I + 98 Da adduct	28176.7	28178.0 ± 4.0	

[a] Amino acid sequences and post-translational modifications were used to obtain the calculated MW values (MW_{calc}). The experimental MW values were measured using MALDI and ESI-MS.

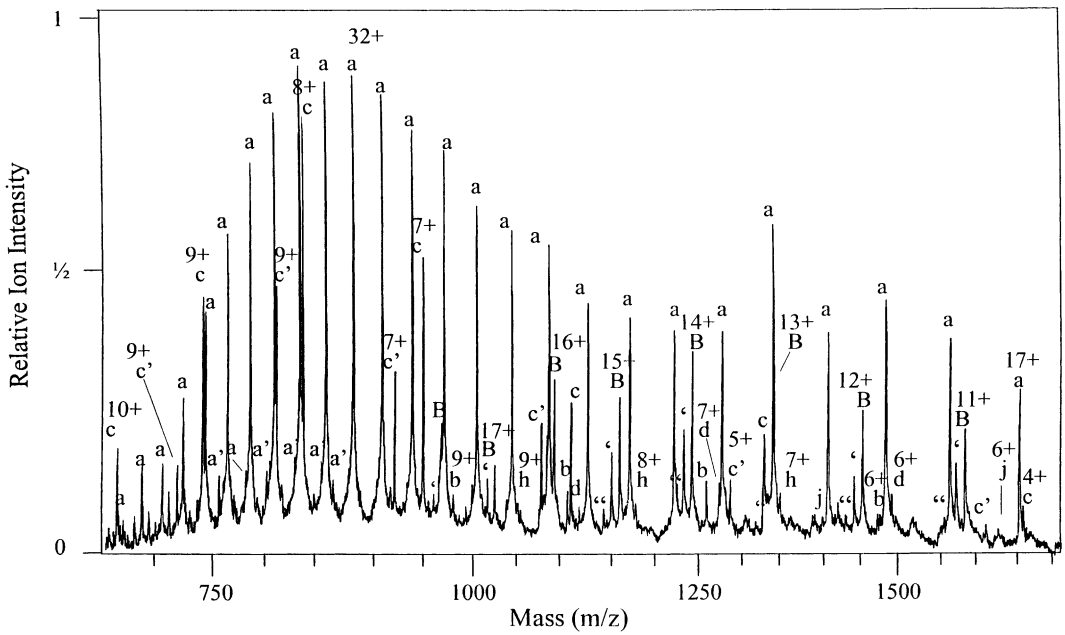

Fig. 2. Positive ion ESI mass spectrum of human serum apolipoproteins from delipidated HDL. The positive numbers identify the number of attached protons and, respectively, the charge of the molecular ions. The one letter code system for the apo isoforms is *c'* = truncated apoC-I, *b* = single chain apoA-II, *d* = proapoC-II. *h* = apoC-III$_1$, *j* = apoC-III$_2$, B" truncated apoA-II, *B'* = truncated apoA-II, *B* = double-chain apoA-II, *a'* = truncated apoA-I, *a* = apoA-I.

4.3. New truncated isoform of apoA-I

On the low mass side of the apoA-I peak in Fig. 1b, a small peak (labelled A-I') was consistently present in samples taken from different sources and on this basis was taken as evidence that it is a truncated isoform of apoA-I present as a normal component of HDL. The MW was measured to be 27,953 ± 9 Da. This species was also observed in the ESI spectrum (Fig. 2, labelled *a'*). The MW difference compared to apoA-I is −128.1 ± 0.8 Da. The C-terminal amino acid for ApoA-I is glutamine (gln) (MW 128.0) suggesting that this isoform is due to in vivo cleavage of this residue.

4.4. New truncated isoforms of apoA-II

The apoA-II peaks in the MALDI spectrum are labelled in Fig. 1b as apoA-II, II' and II". These same ions were detected in the ESI-MS spectrum (Fig. 2, labelled *B*, *B'* and *B"*). Following the same

Table 2
Structural and analytical characteristics of human serum apolipoproteins: number of the individual basic amino acid residues, total number of basic amino acid residues including the N-terminal amino group, MW values and experimentally-determined observed maximum charges of apos in the ESI mass spectrum

Apo	Arg (*R*)	Lys (*K*)	His (*H*)	Total number (+1)	Maximum charge observed	MW	p*I*
ApoC-I	2	9	0	12	11	6630.6	6.5
ApoC-II	1	6	0	8	8	8914.9	5.0
ApoC-III	2	6	0	9	9	~9500	4.8–5.1
ApoA-I	17	22	6	46	42	28078.6	5.54
ApoA-II	4	20	0	25	18	17379.8	4.90

procedure of measuring mass differences and correlating with terminal residues, we identified apoA-II' as double chain apoA-II without a C-terminus gln (ΔMW -128.1 Da) and apoA-II" as double-chain apoA-II with loss of C-terminal gln from both chains ($\Delta MW = -255.0 \pm 1.0$ Da).

4.5. New type of isoform of apoA-II

Two of the prominent peaks in the MALDI spectrum have *m/z* values that are close to each other and do not correspond to any of the known apos. The MW value for the heavier peak is 8809.3 ± 0.6 Da by ESI-MS and 8809.9 ± 0.5 Da by MALDI. The close proximity of these values to that of single-chain apoA-II (MW 8689.9 Da) suggested that these species were composed mainly of single-stranded apoA-II but with a modification that increases the mass by 119.0 ± 0.5 Da. One scenario consistent with the MW values is that it is a single strand of apoA-II formed by a chain of events equivalent to cleavage of the disulfide link between the two strands followed by cysteinylation at the cys_6 cleavage site. This modification would increase the MW of the monomer by the observed 119 Da increment. A-II monomer' would then be the result of truncation of the C-terminal gln on the monomer as was observed with the double chain form.

To test this hypothesis, we carried out a performic acid oxidation using a protocol developed and studied by Sun and Smith [12] and described in the Section 3. Under these conditions, cys, met, and trp are the only residues that undergo MW changes during formic acid oxidation. In the case of the -S-S- bridge between the two apoA-II chains, the -cys-S-S-cys- link is cleaved and converted to two cys-SO_3H residues resulting in an increase by 48 Da in the monomer MW compared to the reduced form (-cys-SH). Methionyl residues are oxidized to the sulfoxide and sulfone producing a mass increase of 16 and 32 Da, respectively. ApoA-II contains no trp residues so it does not contribute to the performic acid oxidation. Fig. 3a and b is the MALDI spectra obtained before and after performic acid oxidation. Fig. 4a brackets the *m/z* region that includes the double-stranded dimer and the putative cysteinylated monomer and its truncated isoform. The amino acid sequences of single and double-stranded apoA-II in the region of the disulfide link are included. The amino acid sequence for the monomer shows the

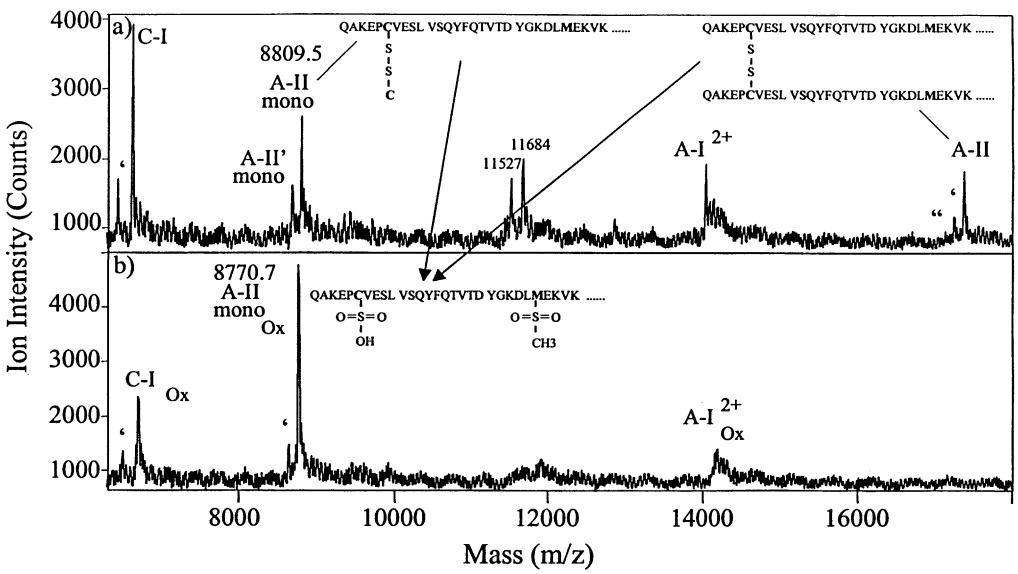

Fig. 3. Positive ion MALDI mass spectrum of human serum apolipoproteins from delipidated HDL: before performic acid oxidation (a) and after performic acid oxidation (b).

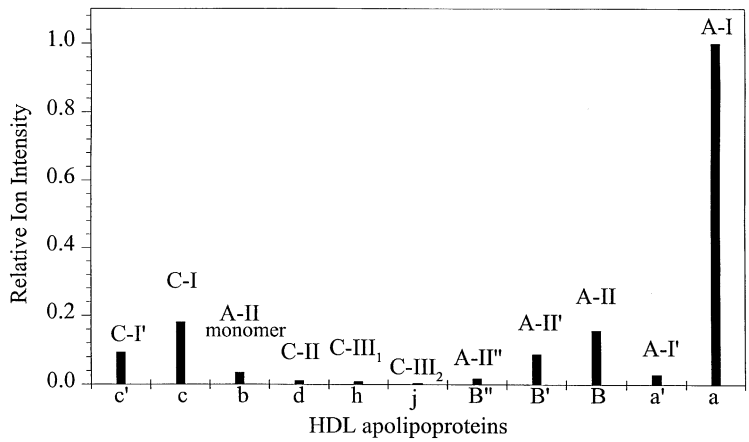

Fig. 4. The deconvoluted ESI mass spectrum of human serum apolipoprotein from the delipidated HDL fraction. Peak heights from all multiply-charged ions for each species in Fig. 2 were added together to produce the histogram and normalized to apoA-I.

postulated residual cys attached to the -S-S- group. Fig. 3b is the spectrum after performic acid oxidation. The peak for the double-stranded apoA-II has disappeared as expected due to oxidation and cleavage of the -S-S- link between the two strands. The *m*/*z* 8809.5 peak has also disappeared and a new peak with high intensity is detected at *m*/*z* 8770.7 corresponding to a ΔMW of -38.8 ± 0.2 Da. In the performic acid oxidation, the cys residue at the disulfide link is lost (−120.1 Da), and an -OH and two oxygens are added (+49 Da). The met residue is converted to a sulfone with the addition of two more oxygen atoms (+32 Da). The net change anticipated in MW is (−120.1 + 49 + 32) or 39 Da, a value consistent with what was observed. We conclude that the new apoA-II isoform is a single-stranded peptide with a side chain -S-S-cys at the cys_6 position in the peptide.

4.6. Interpretation of the charge state distribution of the major apos

If Dr. Hoppillard were in the audience and we presented this data at a meeting, I'm sure that she would ask about what is determining the charge state distribution and how it correlates with the number of basic residues in the apo; a good chemical mass spectroscopist question. Anticipating this question, we have prepared some background information. The three most important basic residues are arg, lys, and his. The number of these residues for the five major HDL apos is listed in Table 2 and the total number is tabulated. This number is then compared with the maximum observed charge state for each apo. The numbers correlate well. This good correlation implies that much of the solution chemistry of this apo mixture is preserved as the charged electrospray droplet evaporates down to the protonated apo. It also suggests that there is little proton transfer between the different apos in the process.

4.7. Relative ion intensities and molar concentrations

The final issue that an inquiring chemist might address is the link between ion intensity in the mass spectrometer and molar concentration in a mixture of proteins. It has already been established that complex charge-transfer reactions take place in the plume generated by the laser pulse. In the MALDI of the HDL apos, the apoC-I intensity dominates even though it is present at a considerably lower concentration than apoA-I. Fig. 4 shows a histogram of the sum of the intensities of all the charge states in the ESI-MS spectrum for an apo relative to the summed inten-

sity for apoA-I. These relative intensities come close to reflecting the concentrations of these species in solution. For example, as discussed in the Section 2, the molar concentration of apoA-I is of the order of 5×10^{-5} M. The apoC-I concentration is five times smaller. The integrated intensities of apoC-I + apoC-I' are approximately four times smaller than apoA-I, in reasonable agreement with the solution concentrations. In contrast, the intensity of apoC-I in the MALDI spectrum, plotted on the same mass scale is four times larger than the apoA-I intensity. The reason for most of this difference is related to the mass dependence ion detection efficiency. This effect has been studied in considerable detail in previous studies [13,14]. When the intensity of the apoA-I peak is corrected for ion detection efficiency, the relative intensity of this peak, compared to the apoC-I intensity, reasonably reflects their relative solution concentrations. But when this analysis is eventually converted to a quantitative measurement, internal standards for each of the apos will have to be incorporated.

5. Summary remarks

MALDI and ESI-MS are now part of the emerging field of proteomics. And proteomics is entering medicine as an important new paradigm in clinical diagnosis. The spectra presented here show how MALDI and ESI-MS working in a complementary mode give information on the protein domains of the lipoproteins. While both methods have evolved from the disciplines of physics, the final product, the gas phase molecular ion, depends on chemistry, gas phase chemistry for MALDI and solution phase chemistry for ESI. The HDL apos have offered an interesting comparison of the two regimes. Dr. Hoppillard has the advantage in her career of being surrounded by the physics and chemistry of mass spectrometry and this is part, but not all of her success in the field and her enormous influence in French mass spectrometry. It's hard to believe that Dr. Hoppillard is old enough to be honored by a special edition of IJMS and we are grateful for the opportunity to participate in the celebration.

Acknowledgements

We are grateful to Steven Cockrill, Layle Watkins, and Ingrid Cruzado for their contributions to the apolipoprotein chemistry part of the project. The expert support in the MALDI measurements provided by Dr. David Russell, Ricky Edmondson, Sharon Shields, and Damon Barbacci was essential for the quality of the data obtained. Dennis Shelton and Mark Miller provided the electronics and computer support and Tony Montalbano, Ken Greer, and Jimmy Hanhart showed a high level of devotion and expertise in precision machining required for the ESI instrument. This work was sponsored by the National Institutes of Health (GM 26096 and HL 54566) and the Robert A. Welch Foundation (A- 258).

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