Supplemental Information

Table S1. Deuterium incorporation rate into different regions of apoA-I in A-I dHDL.

Peptic Peptide	No. of residues	m/z[a] *(charge state)*	Location in the sequence[b]	Time taken for (90-100)% deuterium incorporation	Deuterium incorporation rate[c]
1-13	13	523.60 *(3)*	N-term	15 min	moderate
1-16	16	618.65 *(3)*	N-term	15 min	moderate
3-13	11	662.85 *(2)*	N-term	15 min	moderate
17-28	12	455.93 *(3)*	N-term	15 min	moderate
19-28	10	552.30 *(2)*	N-term	15 min	moderate
29-44	16	877.45 *(2)*	N-term/helix 1	1 min	fast
29-46	18	665.71 *(3)*	N-term/helix 1	1 min	fast
33-46	14	506.65 *(3)*	N-term/helix 1	1 min	fast
34-44	11	565.32 *(2)*	N-term/helix 1	5 min	fast
34-46	13	457.63 *(3)*	N-term/helix 1	1 min	fast
45-50	6	394.74 *(2)*	helix 1	15 min	moderate
45-56	12	689.85 *(2)*	helix 1	0.5 min	fast
47-50	4	547.25 *(1)*	helix 1	0.5 min	fast
61-71	11	652.34 *(2)*	helix 1/helix 2	15 min	moderate
93-103	11	638.36 *(2)*	helix 3/helix 4	180 min	slow
104-111	8	561.79 *(2)*	helix 4	1 min	fast
114-124	11	458.29 *(3)*	helix 4/helix 5	15 min	moderate
115-124	10	420.60 *(3)*	helix 4/helix 5	180 min	slow
115-126	12	501.31 *(3)*	helix 4/helix 5	180 min	slow
127-147	21	807.09 *(3)*	helix 5/helix 6	180 min	slow
137-147	11	621.84 *(2)*	helix 5/helix 6	180 min	slow
137-148	12	687.36 *(2)*	helix 5/helix 6	180 min	slow
148-158	11	433.24 *(3)*	helix 6	960 min	slow
151-158	7	448.31 *(2)*	helix 6	180 min	slow
159-169	11	651.33 *(2)*	helix 6/helix 7	1 min	fast
159-170	12	707.87 *(2)*	helix 6/helix 7	1 min	fast
162-169	8	466.27 *(2)*	helix 6/helix 7	1 min	fast
171-178	8	492.33 *(2)*	helix 7	180 min	slow
179-189	11	579.32 *(2)*	helix 7/helix 8	1 min	fast
181-189	9	479.29 *(2)*	helix 7/helix 8	1 min	fast
190-202	13	486.59 *(3)*	helix 8	15 min	moderate
204-211	8	422.24 *(2)*	helix 8/helix 9	180 min	slow
206-211	6	314.20 *(2)*	helix 8/helix 9	15 min	moderate
213-219	7	407.76 *(2)*	helix 9	180 min	slow
214-219	6	350.25 *(2)*	helix 9	180 min	slow
226-229	4	480.29 *(1)*	helix 10	5 min	fast
230-233	4	403.27 *(1)*	helix 10	15 min	moderate
234-243	10	418.57 *(3)*	helix 10	15 min	moderate
236-243	8	498.29 *(2)*	helix 10	0.5 min	fast

[a]m/z values are the average values of non-deuterated peptic peptides from three independent particle measurements.
[b]Putative helices are numbered using the typical nomenclature (1). N-terminal 43 residues are termed as N-term.
[c]Peptic peptides that almost fully exchanged within 5 min time interval are marked as “fast”; 15 min as “moderate”; and 180 min or above as “slow”.

Table S2. Acetylated apoA-I peptides identified in A-I dHDL and A-I/A-II dHDL.

Peptide notation[a]	Peptide location in apoA-I	Acetylated Lys residues	m/z *(charge state/s)* [b]
12	3-13	PPQSPWDRV**K**D	683.84 *(2)*
23(1)	14-24	LATVYVDVL**K**D	639.37 *(2)*
23(2)	21-34	VL**K**DSGRDYVSQFE	842.93 *(2)*
40-45	35-48	GSALG**K**QLNL**K**LLD	777.47 *(2)*
59(1)	52-62	SVTSTFS**K**LRE	648.86 *(2)*
59(2)	49-62	NWDSVTSTFS**K**LRE	856.44 *(2)*
77(1)	71-78	FWDNLE**K**E	561.77 *(2)*
77(2)	77-85	**K**ETEGLRQE	566.29 *(2)*
88(1)	86-89	MS**K**D	522.22 *(1)*
88(2)	86-91	MS**K**DLE	764.36 *(1)*
94-96(1)	93-102	V**K**A**K**VQPYLD	622.87 *(2)*
94-96(2)	93-103	V**K**A**K**VQPYLDD	680.38 *(2)*
106-107(1)	104-111	FQ**KK**WQEE	603.81 *(2)*
106-107(2)	103-111	DFQ**KK**WQEE	661.32 *(2)*
118(1)	114-125	LYRQ**K**VEPLRAE	515.30 *(3)*
118(2)	112-125	MELYRQ**K**VEPLRAE	601.99 *(3)*
118(3)	114-128	LYRQ**K**VEPLRAELQE	638.70 *(3)*
133	129-136	GARQ**K**LHE	490.78 *(2)*, 327.52 *(3)*
140(1)	140-147	**K**LSPLGEE	914.50 *(1)*, 457.76 *(2)*
140(2)	140-150	**K**LSPLGEEMRD	658.84 *(2)*
182	180-191	AL**K**ENGGARLAE	635.85 *(2)*
195	192-198	YHA**K**ATE	431.22 *(2)*
206-208(1)	206-212	**K**A**K**PALE	420.76 *(2)*
206-208(2)	206-223	**K**A**K**PALEDLRQGLLPVLE	1037.61 *(2)*
226(1)	224-234	SF**K**VSFLSALE	635.36 *(2)*
226(2)	224-235	SF**K**VSFLSALEE	699.87 *(2)*

[a]Peptide notations are as in Figure 7.
[b]m/z values are the average values from three independent particle measurements. Acetylation of a single Lys adds 42.01 Da to a peptide mass.

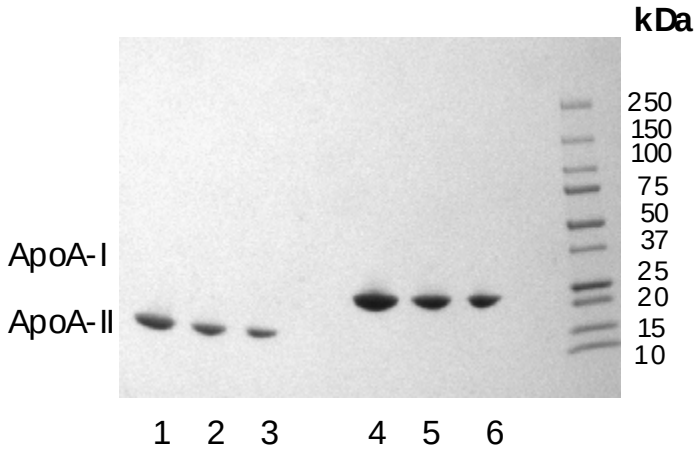

Figure S1. Different Coomassie Blue staining capacities of apoA-I and apoA-II. ApoA-I and apoA-II were separated on a 4-20 % SDS-PAGE (using a Mini-Protein precast gel, Bio-Rad, Hercules, CA). Lanes 1, 2 and 3 were loaded with 10, 5 and 2.5 μg of apoA-II respectively. Lanes 4, 5 and 6 were loaded with 10, 5 and 2.5 μg of apoA-I respectively. The protein concentrations were determined by a standard Markwell-Lowry protein assay (2). The gel was stained with Coomassie Blue. Molar ratio of apoA-I: apoA-II (2:1) used in A-I/A-II dHDL particle preparation are similar to the protein amounts on lane 4 (apoA-I) and lane 3 (apoA-II), respectively.

Supplemental Procedures

HDX followed by sample processing for mass spectrometry analysis: Deuterium incorporation into dHDL (in 5 mM phosphate buffer, pH 7.4, at 4 mg/ml concentration) was initiated by diluting 2.5 µl particle solution to 25 µl by introduction of 5 mM deuterated phosphate buffer (pD 7.4) (3). The sample solutions were maintained at ambient temperature for specific time intervals (0.5 min, 1 min, 5 min, 15 min, 180 min and 960 min), and the reactions were quenched by addition of 5 µl of 0.6 % tri-fluoro acetic acid (Pierce, Rockford, IL). This step lowers the pH to 2.4 ± 0.05, the optimum pH reported to minimize the back exchange of protein incorporated deuteriums. Right after quenching, the sample vials were transferred to a slurry of dry ice/ethanol for quick freezing and then were stored at -70 ^{o}C. Exchanged samples were used within 48 h. We confirmed that the samples stored for 48 h time period had the same amount of incorporated deuteriums as freshly exchanged samples, by subjecting them side by side to mass spectrometry analysis. Deuterium unincorporated control samples (labeled as 0 min) were processed similarly, but by introducing non deuterated phosphate buffer in the place of deuterated phosphate buffer. The "fully deuterated" samples (960 min exchange time point) were prepared by exchanging the samples with 5 mM deuterated phosphate buffer containing 2 M urea to totally denature the protein. Monobasic sodium phosphate, dibasic sodium phosphate and urea used in deuterated buffer preparations were pre-exchanged three times with D_2O followed by lyophilization, to eliminate trapped moisture and/or exchangeable hydrogens that would interfere with data interpretations. D_2O used in the buffer preparations was taken directly from new ampoules (Sigma-Aldrich, St. Louis, MO).

Just before subjecting to mass spectrometry measurements, samples were thawed on ice and were subjected to peptic digestion (pepsin-porcine, Princeton Separations, Adelphia, NJ) on ice with 1:1 (w/w) protein: pepsin ratio for 5 min. Methods were set up so that samples were loaded onto the column exactly at 5.0 min ± 3 sec from the initiation time point of the digestion.

Liquid chromatography-mass spectrometry measurements on peptic digests: Peptide mass detection was carried out on an Applied Biosystems-Sciex QStar XL mass spectrometer equipped with an ion spray source upon separation of the peptides on a capillary high performance liquid chromatography (HPLC, Agillent 1100) system. Sample amounts, 30 pmols, were used per run. The HPLC separation was carried out on a C18 capillary column (0.5 mm id. x 15 cm length, 300 Å particle size, from Grace, Deerfield, IL) using a gradient of acetonitrile from 2% to 40% at a flow rate of 20 µl/min over 12 min. The gradient was generated using solvent A (0.1% formic acid and 2% acetonitrile in water) and solvent B (0.075% formic acid and 2% water in acetonitrile). For manual sample injection, a six port, two position switch valve (Rheodune, Rohnert Park, CA) equipped with a 5 µl sample loop was interfaced between the HPLC output and the column inlet. All the tubing, the switching valve and the column were immersed in ice/water bath to minimize the back exchange of the incorporated deuteriums into the protons.

Due to the short length of the gradient, simultaneous MS/MS sequencing of all candidate peptic peptides was not possible from a single MS run. Hence, we generated a list of all theoretical peptic peptide masses of apoA-I and mapped them with experimental masses (within 50 ppm) generated from an LC-MS of A-I dHDL peptic digest. The same sample used to generate the experimental masses was re-measured with enabled MS/MS function by including a set of experimental masses that eluted at least 30 sec apart from each other, as a list of MS/MS candidates. Once MS/MS sequencing of these peptides was completed and accepted or rejected as a peptide generated from apoA-I, another set of candidate masses were subjected to MS/MS in a subsequent run. The process was carried out till all masses in the experimental list were covered and all peptides were sequenced.

HDX data analysis. Once the identity of an unexchanged peptide was confirmed by MS/MS, its elution time and the charge state were used as a guide to detect its deuterium incorporated forms. The centroids of individual isotopic peak clusters corresponding to their average MW upon deuterium incorporation were selected using HX Express β-version (4) or by manually calculating the centroid using the weighted average of the peak cluster. The average number of deuteriums incorporated into each peptide at time points 0.5 min, 1 min, 5 min, 15 min, 180 mins, were calculated using the equation [1] incorporating a correction to account for the loss of deuteriums during digestion and separation steps as reported before (5,6). In the equation [1], m_{100}% and m_0% correspond to the centroid masses of 100% exchange time point and unexchanged time point respectively, while D% indicates percent deuterium incorporation.

$$D\% = \frac{(m - m_0\%)}{(m_{100}\% - m_0\%)} x100 \qquad [1]$$

Acetic anhydride modification of Lys residues. Partial acetylation of Lys residues on apolipoproteins in dHDL (20mM PBS, pH 7.4) was carried out by introducing fresh non-deuterated acetic anhydride (Ac_2O-d_0, Sigma-Aldritch, St. Louis, MO) in a molar ratio of 1:10 Lys:Ac_2O-d_0. The reaction was continued for 10 min at ambient temperature, while maintaining the pH in the range of 7.4-7.6. The reaction was quenched by the addition of 10% TFA (Pierce, Rockford, IL) to adjust the pH to 3, the acidity required to completely hydrolyze any unreacted Ac_2O-d_0. This was followed by incubation of the reaction mixture for 60 min at ambient temperature. Next, the pH was readjusted to pH 7.4 and the partially acetylated dHDL was dialyzed into 10 mM ammonium bicarbonate followed by lyophilization. Lyophilized samples were delipidated using Bligh-Dyer lipid extraction (7), followed by complete denaturation of proteins with the addition of 100 µl of 3M Guanidine in 50% saturated sodium acetate buffer. At this step, the sample solutions were incubated at 50 ^oC for 60 minutes. Denaturated proteins were subjected to complete acetylation by introducing 10 µl deuterated acetic anhydride (Ac_2O-d_6, Isotec, Miamisbury, OH), by maintaining the pH between 7.4 and 7.6 for 30 min, at ambient temperature. Upon dialyzing into 10 mM ammonium bicarbonate, 50 µg of protein aliquots were subjected to digestion with sequencing grade endoprotenase (Glu-C, Roche Diagnostics, Indianapolis, IN) at 10: 1 protein:Glu-C ratio for 2h at 24 ^oC. The digestion process was repeated once. Digested samples were lyophilized and stored at -20^oC for MS analysis. Positive controls were processed exactly as explained above, except that in the second step, Ac_2O-d_0 was added in the place of Ac_2O-d_6.

Liquid Chromatography and mass spectrometry measurements of Glu-C digests. The mass spectrometer, solvent system and column used were the same as for HDX experiments above. The MS measurements were carried out using sample amounts that ranged from 20-30 pmols and the HPLC separation was carried out using a linear gradient of acetonitrile from 2% to 40% at a flow rate of 6 µl/min over a 120 min time period. Automated data acquisition was carried out using information dependent acquisition (IDA) functionality of the Analyst QS software. Each MS spectrum (acquired in 1 s) was followed by acquisition of three MS/MS spectra of the three most intense ions after satisfying the dynamic exclusion criteria. This criterion was set up in a way that a peptide mass already fragmented within a 60 s window with a mass tolerance of 100 ppm would not get fragmented again within that time period.

Data analysis. Upon completion of the mass spectrometry runs, MS/MS spectra were subjected to Mascot searches (Matix Science) to confirm the identity of unmodified peptides and acetylated peptides. Once confirmed, extracted ion chromatograms corresponding to acetylated peptides were manually analyzed and the intensity of the monoisotopic peaks originated from Ac_2O-d_0 modification and Ac_2O-d_6 modification were separately recorded. The percent solvent exposure of Lys residue(s) in a peptide was calculated as reported before using the equation [2], where Int(Ac_2O-d_0) and Int(Ac_2O-d_6) are the intensities of the monoisotopic peaks originating from Ac_2O-d_0 and Ac_2O-d_6 modifications of Lys residues respectively (8).

$$\text{Solvent exposure} = \frac{Int(Ac_2O - d_0)}{Int(Ac_2O - d_6) + Int(Ac_2O - d_0)} x100 \qquad [2]$$

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