Supporting Information

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SI Text

Preparation of Methionine Oxidized apoA-I. ApoA-I (~9 mg/ml) was oxidized by incubation with 0.3% hydrogen peroxide in 100 mM sodium phosphate, pH 7.4 at 37 °C for 8 h. This procedure was modified from previously described methods (S1, S2) and was found to oxidise the methionines of apoA-I while minimizing alternative oxidation products. Oxidized apoA-I was separated from excess hydrogen peroxide by buffer exchange into 100 mM sodium phosphate, pH 7.4. ApoA-I with all three methionine residues oxidized (MetO-apoA-I) was purified by preparative HPLC on a C_4 column. The purity of the product was assessed by HPLC and mass spectrometry (Fig. S1).

Methods. ***Mass spectrometry.*** Samples of apoA-I and MetO-apoA-I were dialysed against 10 mM ammonium bicarbonate prior to analysis. Mass spectrometry was performed on an Agilent Quadrupole time of flight (Q-TOF) instrument (Palo Alto, CA) equipped with an ESI source. Freshly prepared protein samples (10 μl) were injected at a flow rate of 250 μl/min via a line containing 0.1% formic acid in 25% acetonitrile. Mass spectral data were deconvoluted and analyzed using the program Mass Spectrum Analyser (Agilent). Tandem mass spectrometry was performed by digestion of MetO-apoA-I (0.4 mg/mL) with trypsin (5 μg/mL) in a total volume of 200 μl, followed by online liquid chromatography tandem mass spectrometry on an Agilent Q-TOF. The MS/MS data were analyzed and interpreted using Spectrum Mill and the Agilent Mass Hunter software (Agilent).

MetO-apoA-I fibrils were pelleted by centrifugation at 14,000 *g* for 5 min and resuspended in an equal volume of 10 mM ammonium bicarbonate. Resuspension was repeated 3 times to remove buffer salts and the final pellet was resuspended in 100% acetonitrile to dissociate fibrillar material. Time of flight mass spectrometry was performed and data were analyzed as described above.

Circular dichroism spectroscopy. CD spectra were recorded for apoA-I and MetO-apoA-I (0.2 mg/mL) by using a Jasco J-815 CD spectrometer (Tokyo) equipped with a Peltier temperature control module between 190–250 nm at 20 °C, using a 1 mm pathlength quartz cuvette, 0.5 nm step size, 1 nm bandwidth, and 2 s averaging. Raw spectra were corrected for buffer contribution and converted to mean residue ellipticity. Proteins were freshly prepared and kept on ice until immediately before use. UV-Vis absorbance spectra were recorded before and after acquisition of CD spectra to assess accurate protein concentration and to confirm the absence of protein aggregation.

For thermal denaturation studies, apoA-I and MetO-apoA-I (0.2 mg/mL) were prepared in 100 mM sodium phosphate buffer, pH 6.0. CD measurements were taken at 222 nm with 2 s averaging in a 1 mm pathlength quartz cuvette. A temperature range from 4 to 90 °C was used with 1 °C increments allowing 1 min for temperature equilibration.

Both apoA-I and MetO-apoA-I appeared to unfold via a two-state mechanism. Melting temperatures (T_m) were estimated by fitting data to a two-state transition model by using the program SigmaPlot 9.0 (SPSS Ltd, UK) as previously described (S3).

Far UV CD spectra for MetO-apoA-I fibrils (0.12 mg/mL) were recorded using an Aviv 410SF CD spectrometer (NJ) between 190–250 nm at 20 °C, using a 1 mm pathlength quartz cuvette, 0.5 nm step size, 1 nm bandwidth and 6 s averaging. Raw spectra were corrected for buffer contribution and converted to mean residue ellipticity. MetO-apoA-I fibrils were pelleted by centrifugation at 14,000 *g* for 5 min and resuspended in 100 mM sodium phosphate buffer, pH 6.0 prior to analysis.

Analytical ultracentrifugation. Analytical ultracentrifugation experiments were performed in a Beckman XL-I analytical ultracentrifuge equipped with UV/Vis scanning optics, An-60 Ti 4-hole rotor, and double-sector cells with quartz windows (Beckman Coulter, Inc., CA).

ApoA-I and MetO-apoA-I were prepared at 0.02–1.6 mg/mL in 100 mM sodium phosphate buffer, pH 6.0. Sample and reference solutions were loaded into 12 mm double-sector cells with quartz windows and centrifuged at 40,000 rpm and 20 °C. Radial absorbance data were collected at wavelengths of between 230 and 295 nm, according to the absorbance signal at each protein concentration, with radial increments of 0.003 cm and radial scans were recorded at 6 min intervals. Sedimentation coefficient (c[s]) distributions (S4) were normalized with respect to integrated signal intensity separately for apoA-I and MetO-apoA-I.

Thioflavin T fluorescence measurements. ApoA-I and MetO-apoA-I (0.3, 0.5, and 0.8 mg/ml) were incubated at 37 °C in the presence of 10 μM ThT in 100 mM sodium phosphate, pH 6.0. At defined time points, 200 μl of each sample were placed in a 96 well plate. Fluorescence was measured in duplicate by using a f_{max} fluorescence plate reader (Molecular Devices, CA) with excitation and emission filters of 444 and 485 nm, resp.

Congo Red staining. Congo Red stain was prepared as a saturated solution in 80% ethanol, 20% distilled water, saturated NaCl as described previously (S8). 100 μl of stain was added to 10 μl of MetO-apoA-I fibril suspension (0.8 mg/mL) and incubated for 2 min. Fibrils were washed 3 times by centrifugation at 14,000 *g* for 5 min and resuspension in 50 μl distilled water. Stained fibrils were finally resuspended in 10 μl distilled water. 2 μl drops of the final suspension were placed on poly-lysine coated slides and dried at 37 °C for 1 h.

Samples were viewed under bright-field and cross-polarized light on an Olympus BX51 light microscope and images were recorded with a SPOT 1.3.0 digital camera (Diagnostic Instruments, MI) at 20 times magnification.

Grid preparation for transmission electron microscopy. Carbon-coated Formvar 300 mesh copper grids were rendered hydrophilic by glow discharge under reduced atmosphere for 10 sec. Suspensions of MetO-apoA-I fibrils were diluted to 0.1 mg/mL protein with distilled water, and 3.5 μl was applied to each grid and allowed to adsorb for 30 sec. Grids were washed twice with distilled water, negatively stained with 2 % aqueous potassium phosphotungstate, pH 6.8, and air dried.

X-ray fibre diffraction studies MetO-apoA-I fibrils grown at 0.8 mg/mL were concentrated to approximately 15 mg/mL by centrifugation at 14,000 *g* for 5 min and resuspension in a small volume of distilled water. Resuspension was repeated 3 times to remove buffer salts. 2–5 μl of concentrated fibril suspension was loaded into siliconised, thin walled 0.5 mm quartz capillaries (Hampton Research CA) and allowed to dry slowly (approximately 1–2 weeks) in the magnetic field of an unshielded 600 MHz NMR spectrometer. The resultant semi-dehydrated samples exhibited strong birefringence under cross-polarized light (Fig. S4) indicating that this protocol had produced alignment in the fibrils.

X-ray diffraction images were acquired at the Australian Synchrotron MX2 micro-focus beamline with the X-ray source operating at 12.658 keV and 20% beam attenuation. The sample to detector distance was 350 mm and the exposure time was 10 s. Background scattering obtained under identical conditions from a section of the quartz capillary containing no sample was subtracted from raw diffraction images prior to analysis

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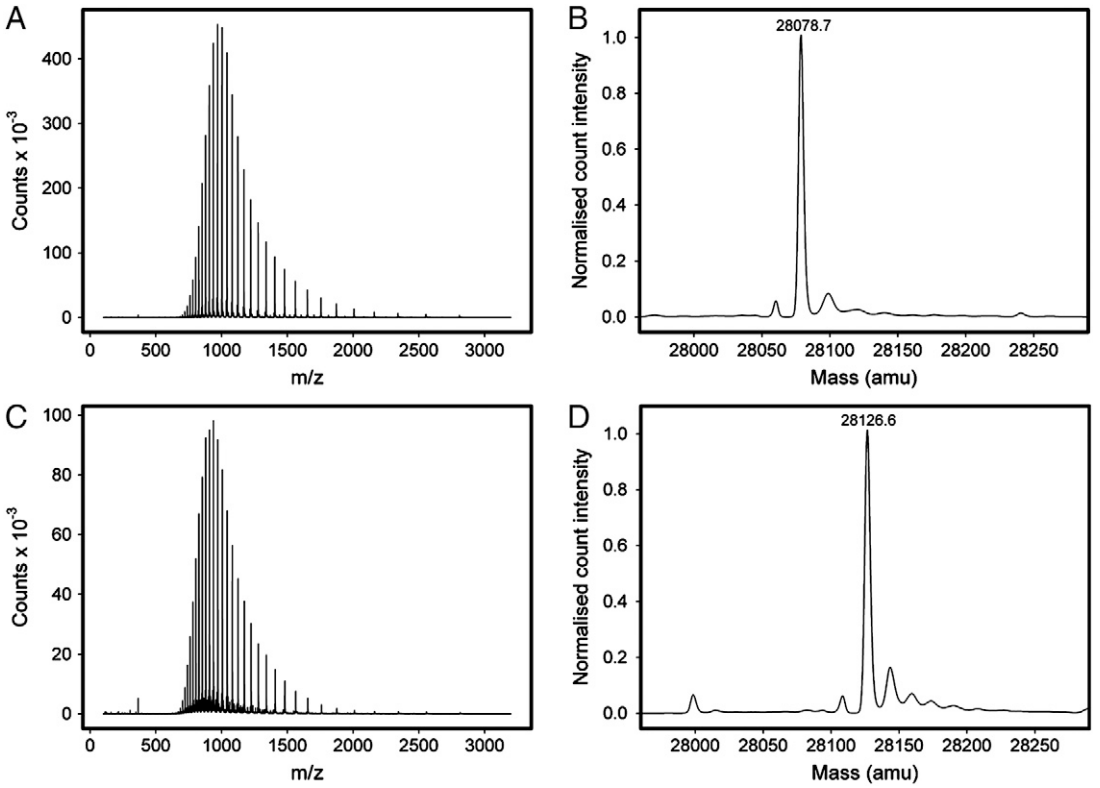

Fig. S1. Mass spectrometry analysis of native apoA-I and MetO-apoA-I. Raw m/z data and deconvoluted mass data are presented for native apoA-I (*A* and *B*) and MetO-apoA-I (*C* and *D*). The mass of purified MetO-apoA-I showed an increase of 48 amu over that of native apoA-I indicating that all three methionines were oxidized to methionine sulfoxide. The series of small peaks at higher mass in *B* and *D* were identified as sodiated adducts of the protein. Tandem mass spectrometry of a trypsin digest of MetO-apoA-I identified each methionine sulfoxide. Other common protein oxidation products were below detectable levels using these methods.

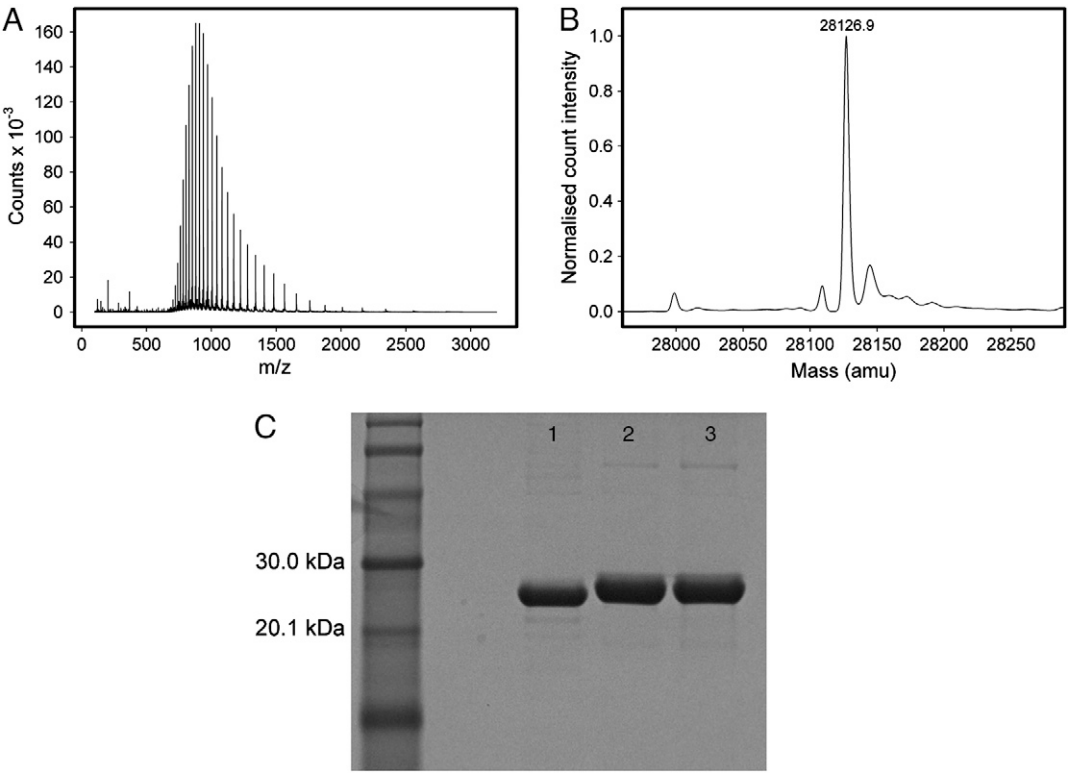

Fig. S2. Mass spectrometry and SDS-PAGE analysis of fibrillar MetO-apoA-I. Fibrils were isolated and dissociated prior to analysis. Raw m/z data and deconvoluted mass data (*A* and *B*) indicated that fibrils were composed solely of MetO-apoA-I containing three methionine sulfoxide residues (native protein mass +48 amu) and no smaller fragments were observed. The series of small peaks at higher mass in *B* were identified as sodiated adducts of the protein. SDS-PAGE analysis (*C*) of native apoA-I (lane 1), MetO-apoA-I before incubation (lane 2), and MetO-apoA-I extracted from isolated fibrils (lane 3) showed no detectable fragmentation of the protein comprising fibrils.

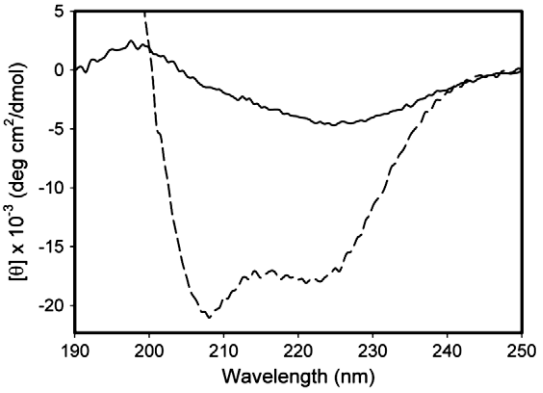

Fig. S3. Far UV circular dichroism spectrum of MetO-apoA-I fibrils (*Solid Line*) and freshly prepared MetO-apoA-I (*Dashed Line*) at pH 6.0 (see also Fig. 1*A*). MetO-apoA-I fibrils showed significantly reduced CD signal intensity, and a shift in the spectral minimum to approximately 224 nm, relative to unincubated MetO-apoA-I. This indicates that the secondary structure within the fibrils is significantly different to that in the unaggregated protein, and is consistent with a shift towards a higher proportion of β-structure in the fibrils. Fibrils were prepared by centrifugal pelleting and resuspension in 100 mm sodium phosphate buffer pH 6.0 prior to analysis.

Andreola et al. reported a similar loss of CD signal intensity and spectral shift on fibril formation by the 1–93 fragment of apoA-I (S6).

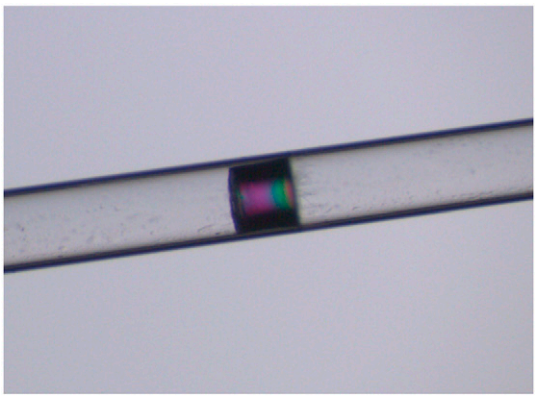

Fig. S4. Magnetically aligned samples of MetO-apoA-I fibrils exhibited strong birefringence under cross-polarized light indicating significant alignment of the fibrils. The diameter of the capillary is 0.5 mm. Other experiments using the stretch-frame method of drying fibril drops (S7) were unsuccessful in producing fibril alignment.

Table S1. Solution conditions examined for incubation of full-length, native apoA-I

	pH	Protein concentration	Temperature	Agitation*	Guanidine.HCl†	Short chain phospholipid‡
		mg/mL	°C		M	mM
1	2.0	0.8	37	no	—	—
2	4.0	0.8	37	no	—	—
3	5.0	0.8	37	no	—	—
4	7.4	0.8	37	no	—	—
5	10.0	0.8	37	no	—	—
6	4.0	0.4	37	yes	—	—
7	5.0	0.4	37	yes	—	—
8	6.0	0.4	37	yes	—	—
9	4.0	0.8	37	yes	—	—
10	4.2	0.8	37	yes	—	—
11	4.4	0.8	37	yes	—	—
12	4.6	0.8	37	yes	—	—
13	4.8	0.8	37	yes	—	—
14	5.0	0.8	37	yes	—	—
15	5.2	0.8	37	yes	—	—
16	5.4	0.8	37	yes	—	—
17	5.6	0.8	37	yes	—	—
18	5.8	0.8	37	yes	—	—
19	6.0	0.8	37	yes	—	—
20	7.4	0.8	37	yes	—	—
21	10.0	0.8	37	yes	—	—
22	4.0	1.6	37	yes	—	—
23	5.0	1.6	37	yes	—	—
24	6.0	1.6	37	yes	—	—
25	5.0	0.8	65	no	—	—
26	7.4	0.8	65	no	—	—
27	7.4	0.8	37	no	—	3
28	7.4	0.8	37	no	—	20
29	7.4	0.8	37	no	0.2	—
30	7.4	0.8	37	no	0.5	—
31	7.4	0.8	37	no	0.75	—
32§	7.4	4.0	37	no	—	—

Fibril formation by native apoA-I was not observed under any of the listed conditions.

*Samples were mixed constantly at 600 rpm in an Eppendorf Thermomixer Comfort orbital shaker.
†ApoA-I was found by CD to be completely denatured at 1 M Guanidine.HCl.
‡1,2-Dihexanoyl-*sn*-Glycero-3-Phosphocholine; 3 mM is submicellar, 20 mM is micellar.
§Conditions reported to yield fibrils by Wisniewski et al. (S5).