SUPPLEMENTAL DATA

Preparation of lipid-free apoA-I for exchange reactions

Because the self-association of lipid-free apoA-I can influence the rate of lipid-protein complex formation (1), lipid free apoA-I:ΔW used for the apoA-I exchange experiments was diluted at ≤ 0.1 mg/ml and denatured with 4.5 M guanidine over-night at room temperature. The protein was allowed to slowly refold at low concentration removing guanidine by three day dialysis versus TBS (8.2 mM Tris, 150 mM NaCl, 1 mM EDTA, pH 7.4) at 4°C, with three buffer changes. The protein was concentrated by Vivaspin-6 10,000 MWCO ultra-filtration devices (Sartorius Biotech Inc., Edgewood, NY) to ≈ 2.0 mg/ml, stored at 37°C and used for exchange experiments within 72 h from refolding. This treatment guaranteed the disruption of high order oligomeric species. During storage at 37°C, high order oligomers were not observed within one week from refolding. Cross-linking analysis and routine analysis by NDGGE showed > 90% of apoA-I:ΔW in monomeric form, comparable to plasma purified apoA-I. SDS-PAGE showed no protein degradation upon treatment (Fig. S1*A*).

FIGURE LEGENDS

Fig. S1. Panel A: non reducing 4-20% gradient SDS-PAGE of recombinant proteins. Molecular weight markers (kDa), lane 1; wild type recombinant apoA-I, lane 2; apoA-I:ΔW, lane 3; apoA-I:ΔW denatured with 4.5 M guanidine, refolded at low concentration (≤ 0.1 mg/ml) and concentrated at 2.19 mg/ml, lane 4; apoA-I:W19-AED136, before and after AEDANS labeling and re-purification, lane 5 and 6, respectively; apoA-I:ΔW oxidized by $ONOO^-$ or MPO-H_2O_2-nitrite treatment, lane 7 and 8, respectively. Gels of 1 mm thickness were used and the amount of protein loaded per lane was 3 μg. The figure represents a composite gel in which lanes 7 and 8 were added from a different electrophoretic run by alignment of the MW markers. Panel B: NDGGE analysis of lipid-free apoA-I:W19-A350-136 incubated at 37°C in the presence (lane 4-7) or absence (lane 3) of a five-fold molar excess of wild-type apoA-I 7.8 nm rHDL particles. rHDL-associated and lipid-free protein were separated by electrophoresis on 4-20% non-denaturing tris-glycine polyacrylamide gels and visualized as described in Fig. 2. Molecular weight markers (High Molecular Weight Calibration Kit from GE Healthcare), lane 1. Lipid-free apoA-I:W19-A350-136 stored at 4°C or incubated at 37°C for 5 h, lane 2 and 3, respectively. Incubation mixtures of lipid-free apoA-I:W19-A350-136 and a five-fold molar excess of wild-type apoA-I 7.8 nm rHDL at 5 h and 24 h, lane 4, 5 (fluorescence) and lane 6, 7 (total protein staining), respectively.

Fig. S2. NDGGE of rHDL particles. Molecular weight markers (High Molecular Weight Calibration Kit from GE Healthcare), lane 1; rHDL of 9.6, 8.4, and 7.8 nm diameters reconstituted using apoA-I:W19-AED136, lane 2-4, respectively; BS^3 cross-linked 7.8 nm rHDL, lane 5.

Fig. S3. Representative NDGGE (4-20% tris-glycine polyacrylamide) gel of wild type apoA-I 7.8 nm rHDL incubated at 37 ºC for 24 h in the presence (lane 4) or absence (lane 3) of a five-fold molar excess of lipid-free plasma-purified apoA-I. 7.8 nm rHDL stored at 4 ºC for 24h, lane 2. Molecular weight markers (High Molecular Weight Calibration Kit from GE Healthcare), lane 1.

Fig. S4. Fluorescence spectra of lipid-free apoA-I variants after SDS treatment, panel A, and of 7.8 nm rHDL (apoA-I:W19-AED136) incubated at 37 ºC, panel B. Panel A, lipid-free apoA-I:ΔW-W19 (black), apoA-I:ΔW-AED136 (red), and apoA-I:W19-AED136 (blue) in the presence of 1% SDS (1h, RT). Instrument setting was as described in Fig. 4. Panel B, fluorescence spectra of 7.8 nm rHDL (apoA-I:W19-AED136) incubated at 37 ºC for up to 72 h. Instrument setting and incubation conditions were as described in Fig. 5. Tyr background and direct excitation of AEDANS were eliminated by subtracting the emission spectra of apoA-I:ΔW-AED136 (acceptor-only in Trp null background) from the emission spectra of apoA-I:W19-AED136.

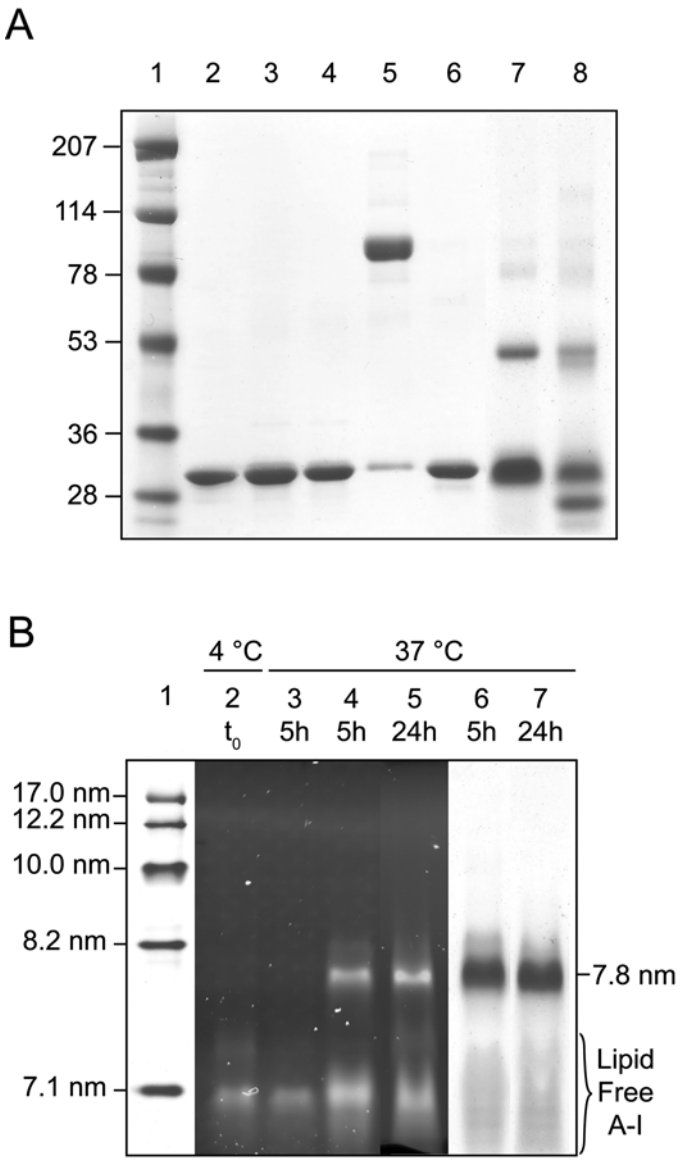
Fig. S5. LC-ESI-MS and MS/MS analysis of apoA-I:ΔW exposed to the MPO-H_2O_2-nitrite system. ApoA-I:ΔW (~20 μM) was exposed to PBS buffer (pH 7.4) alone (control) or 200 μM H_2O_2 in the MPO-nitrite system (50 nM MPO and 150 μM $NaNO_2$) for 1 h at 37 °C (see experimental procedures and (2,3)). Reactions were initiated by adding oxidant and terminated by adding a 10-fold molar excess of L-methionine. ApoA-I:ΔW was then reduced, alkylated, and digested with trypsin, and the tryptic digest was analyzed with LC-ESI-MS and MS/MS. Panel A, reconstructed ion chromatograms of peptides containing unmodified and nitrated Tyr18 in control apoA-I:ΔW and apoA-I:ΔW exposed to the MPO-H_2O_2-nitrite system. Panel B and C, MS and MS/MS analysis of precursor peptide containing unmodified and nitrated Tyr18, respectively. Nitrated Tyr18 peptide was not detected in control apoA-I:ΔW (panel A). Compared with native Tyr18 peptide (panel B), all the y6–y9 and b6–b8 ions had gained 45 amu, whereas b2–b5 and y4-y5 were unmodified (panel C), indicating that Tyr18 in this peptide had been converted to nitrotyrosine (Tyr+45).

Fig. S6. Quantification of oxidized methionine (panel A) and nitrated tyrosine (panel B) in apoA-I:ΔW exposed to $ONOO^-$ or the MPO-H_2O_2-nitrite system (MPO-N). ApoA-I:ΔW (~20 μM) was exposed to 500 μM $ONOO^-$ or 200 μM H_2O_2 in the MPO-nitrite system (50 nM MPO and 150 μM $NaNO_2$) for 1 h at 37 °C in PBS (see experimental procedures and Fig. 5S). After the reaction was terminated with 10 mM L-methionine, apoA-I:ΔW was digested with trypsin or Glu-C, and the peptides were analyzed with LC-ESI-MS/MS. Peptide sequences were confirmed using MS/MS. Oxidized peptides were detected and quantified using reconstructed ion chromatograms of precursor and product peptides (Fig. S5). Product yield of oxidized peptides was calculated as: product yield (%) = [(product ion peak area) / (precursor ion peak area + product ion peak area)] × 100 (3). Results are representative of those from 2 independent experiments.

SUPPLEMENTAL DATA REFERENCES

1. Massey, J. B., Gotto, A. M., Jr., and Pownall, H. J. (1981) *Biochem Biophys Res Commun* **99**(2), 466-474
2. Shao, B., Bergt, C., Fu, X., Green, P., Voss, J. C., Oda, M. N., Oram, J. F., and Heinecke, J. W. (2005) *J Biol Chem* **280**(7), 5983-5993
3. Shao, B., and Heinecke, J. W. (2008) *Methods Enzymol* **440**, 33-63

Figure S1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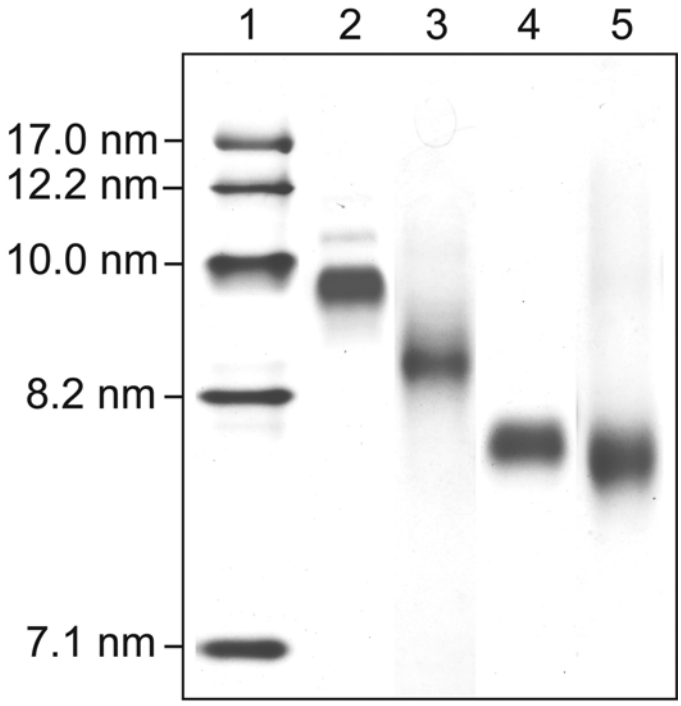
1 2 3 4 5 6 7 8
207
114
78
53
36
28
B
4 °C
37 °C
1 2 3 4 5 6 7
t0 5h 5h 24h 5h 24h
17.0 nm
12.2 nm
10.0 nm
8.2 nm
7.1 nm
7.8 nm
Lipid Free A-I

Figure S2



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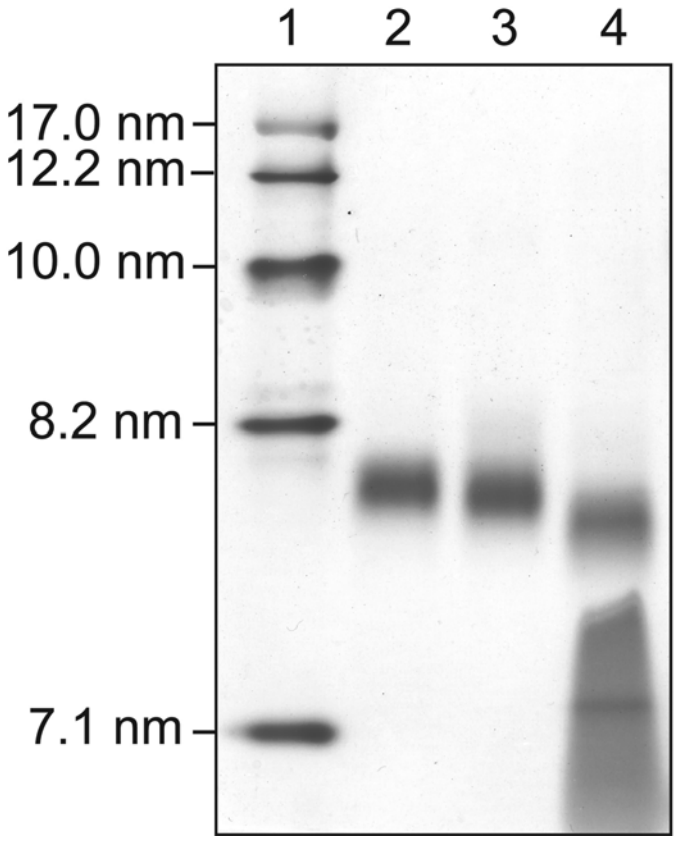


Figure S4

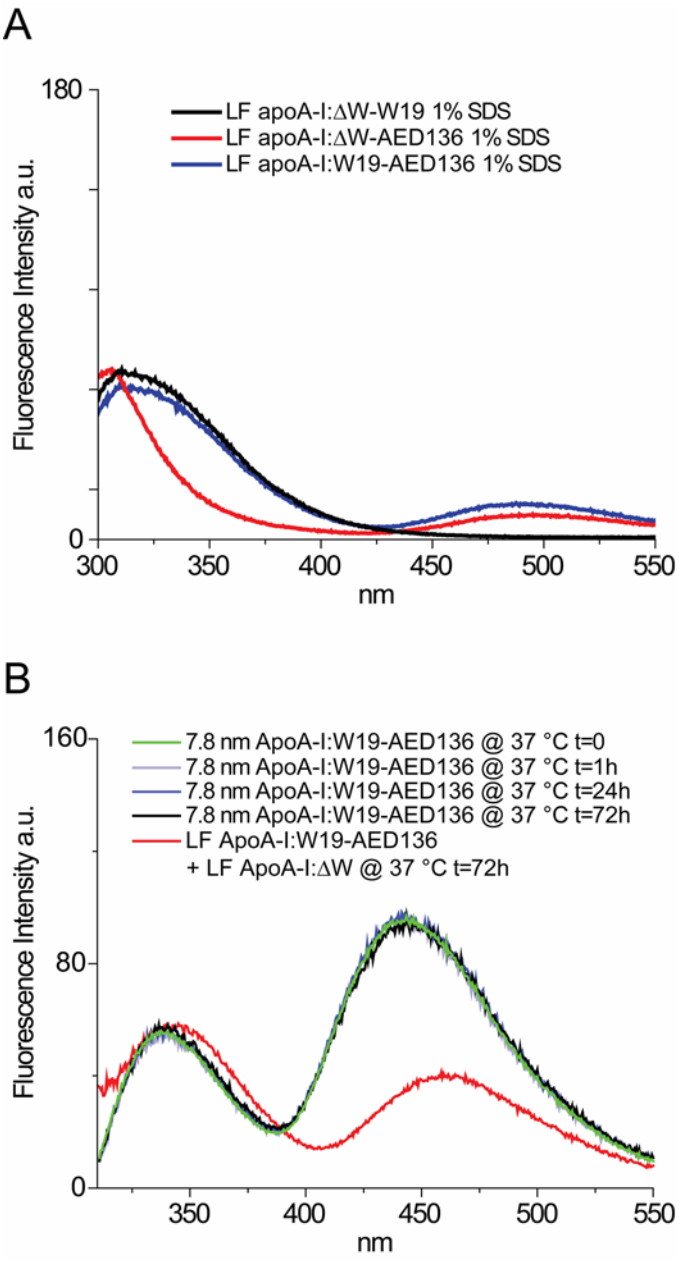
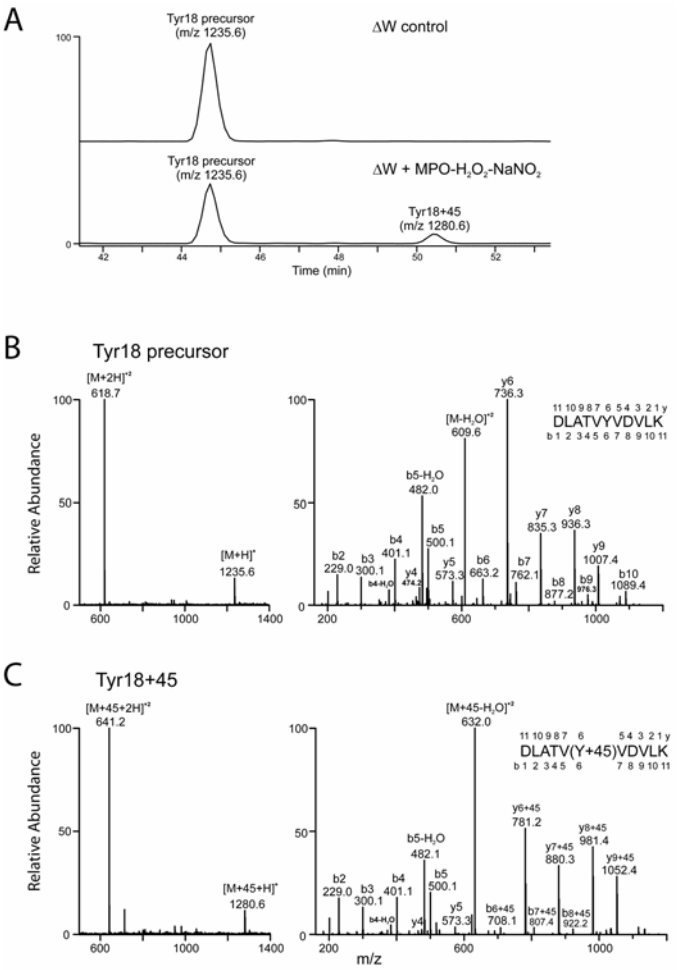

A
180
0
Fluorescence Intensity a.u.
LF apoA-I:ΔW-W19 1% SDS
LF apoA-I:ΔW-AED136 1% SDS
LF apoA-I:W19-AED136 1% SDS
300
350
400
450
500
550
nm
B
160
80
0
Fluorescence Intensity a.u.
7.8 nm ApoA-I:W19-AED136 @ 37 °C t=0
7.8 nm ApoA-I:W19-AED136 @ 37 °C t=1h
7.8 nm ApoA-I:W19-AED136 @ 37 °C t=24h
7.8 nm ApoA-I:W19-AED136 @ 37 °C t=72h
LF ApoA-I:W19-AED136
+ LF ApoA-I:ΔW @ 37 °C t=72h
350
400
450
500
550
nm

Figure S5


A
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(m/z 1235.6)
ΔW control
Tyr18 precursor
(m/z 1235.6)
ΔW + MPO-H2O2-NaNO2
Tyr18+45
(m/z 1280.6)
Time (min)
B
Tyr18 precursor
[M+2H]+2
618.7
[M+H]+
1235.6
Relative Abundance
DLATVYVDVLK
[M-H2O]+2
609.6
y6
736.3
b5-H2O
482.0
y7
835.3
y8
936.3
b5
500.1
b4
401.1
y9
1007.4
b2
229.0
b3
300.1
y4
474.2
y5
573.3
b6
663.2
b7
762.1
b8
877.2
b9
976.3
b10
1089.4
C
Tyr18+45
[M+45+2H]+2
641.2
[M+45+H]+
1280.6
Relative Abundance
DLATV(Y+45)VDVLK
[M+45-H2O]+2
632.0
y6+45
781.2
b5-H2O
482.1
y8+45
981.4
y7+45
880.3
y9+45
1052.4
b5
500.1
b4
401.1
b2
229.0
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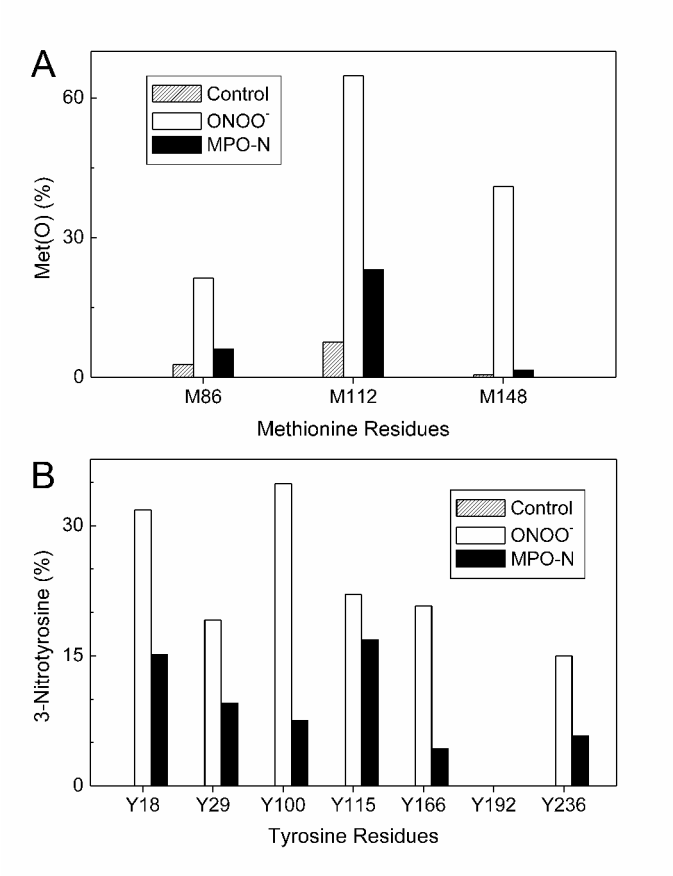
922.2
m/z

Figure S6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MPO-N
Met(O) (%)
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30
0
M86
M112
M148
Methionine Residues
B
Control
ONOO-
MPO-N
3-Nitrotyrosine (%)
30
15
0
Y18
Y29
Y100
Y115
Y166
Y192
Y236
Tyrosine Residues