Supporting Information

Mass Spectrometric Characterization of Protein Modification by the Products of Non-Enzymatic Oxidation of Linoleic Acid

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Table S1. Modified Peptides Detected by LC-ESI-MS/MS from Chymotryptic Digest

Peptide sequence[$]	Position	Mass of modified peptide	Assignment (Mass Shift)
HIRL	146–149	694.4/699.4	HNE–His MA (156/161 Da)
SFNPTQLEEQC*H**H**I	150–162	1758.7/1763.6	
HIRL	146–149	766.4	HODA–His MA (228 Da)
SFNPTQLEEQC*H**H**I	150–162	1830.8	
VEEL**K**PTPEGDLEIL	43–57	1835.8/1840.7	ONE–Lys 4-Ketoamide (154/159 Da)
LQ**K**W	58–61	728.3/733.3	
KIPAVF	77–82	828.3/833.8	
KIDAL	83–87	713.5/718.3	
KIDALNEN**K**VL	83–93	1410.8/1415.9	
NEN**K**VL	88–93	870.3/875.3	
KKY	100–102	592.4/597.3	
K**K**Y	100–102	592.5/597.5	
VRTPEVDDEALE**K**F	123–136	1801.9/1806.8	
D**K**AL	137–140	600.6/605.5	
VEEL**K**PTPEGDLEIL	43–57	1907.6	DODE–Lys 4-Ketoamide (226 Da)
LQ**K**W	58–61	800.4	
KIPAVF	77–82	900.5	
KIDAL	83–87	785.4	
KIDALNEN**K**VL	83–93	1482.8	
NEN**K**VL	88–93	942.3	
KKY	100–102	664.5	
K**K**Y	100–102	664.4	
VRTPEVDDEALE**K**F	123–136	1874.1	
D**K**AL	137–140	672.4	
KALPM	141–145	785.3	
VRTPEVDDEALE**K**F + AC*Q**C**L	123–136 + 118–122	1180.4/1182.9[#]	Cys–ONE–Lys Pyrrole Cross-link (118/123 Da)
VRTPEVDDEALE**K**F + VC*Q**C**L	123–136 + 118–122	1194.3/1196.7[#]	
VRTPEVDDEALE**K**F + **C**L	123–136 + 121–122	1001.0/1003.8[#]	
VRTPEVDDEALE**K**F + AC*Q**C**L	123–136 + 118–122	1216.1[#]	Cys–DODE–Lys Pyrrole Cross-link (190 Da)
VRTPEVDDEALE**K**F + VC*Q**C**L	123–136 + 118–122	1230.3[#]	

VRTPEVDDEALE**K**F + **C**L	123–136 + 121–122	1036.8[#]	
LIVTQ + **H**IRL	1–5 + 146–149	1228.7/1233.6	His–ONE–Lys Pyrrole Cross-link (118/123 Da)
KGL + **H**IRL	8–10 + 146–149	972.5/977.5	
VEEL**K**PTPEGDLEIL + **H**IRL	43–57 + 146–149	1169.3/1172.7[#]	
KIPAVF + **H**IRL	77–82 + 146–149	1329.8/1334.8	
KIDAL + **H**IRL	83–87 + 146–149	1214.6/1219.7	
KIDALNEN**K**VL + **H**IRL	83–93 + 146–149	956. 7/959.3[#]	
KKY + **H**IRL	100–102 + 146–149	1093.4/1098.5	
K**K**Y + **H**IRL	100–102 + 146–149	1093.6/1098.7	
VRTPEVDDEALE**K**F + **H**IRL	123–136 + 146–149	1152.4/1154.7[#]	
D**K**AL + **H**IRL	137–140 + 146–149	1101.6/1106.7	
LIVTQ + **H**IRL	1–5 + 146–149	1301.1	His–DODE–Lys Pyrrole Cross-link (190 Da)
KGL + **H**IRL	8–10 + 146–149	1045.1	
VEEL**K**PTPEGDLEIL + **H**IRL	43–57 + 146–149	1205.4[#]	
KIPAVF + **H**IRL	77–82 + 146–149	1401.6	
KIDAL + **H**IRL	83–87 + 146–149	1286.7	
KIDALNEN**K**VL + **H**IRL	83–93 + 146–149	992.5	
K**K**Y + **H**IRL	100–102 + 146–149	1165.7	
VRTPEVDDEALE**K**F + **H**IRL	123–136 + 146–149	1188.8[#]	
D**K**AL + **H**IRL	137–140 + 146–149	1173.5	
VEEL**K**PTPEGDLEIL	43–57	1817.7/1822.7	ONE–Lys Pyrrolinone (136/141 Da)
LQ**K**W	58–61	710.3/715.2	
KIPAVF	77–82	810.5/815.5	
KIDAL	83–87	695.4/699.4	
KIDALNEN**K**VL	83–93	1392.5/1397.4	
NEN**K**VL	88–93	852.4/857.5	
KKY	100–102	574.3/579.3	
K**K**Y	100–102	574.4/679.3	
VRTPEVDDEALE**K**F	123–136	1783.8/1788.8	
VEEL**K**PTPEGDLEIL	43–57	1889.6	DODE–Lys Pyrrolinone (208 Da)
LQ**K**W	58–61	782.6	
KIPAVF	77–82	882.5	
KIDAL	83–87	767.5	
KIDALNEN**K**VL	83–93	1464.6	
NEN**K**VL	88–93	924.3	
KKY	100–102	646.4	
K**K**Y	100–102	646.4	
VRTPEVDDEALE**K**F	123–136	1855.7	
D**K**AL	137–140	654.2	
KIDALNEN**K**VL	83–93	1354.7/1359.7	N^{ε}-Hexanoyllysine (98/103 Da)
KIDALNEN**K**VL	83–93	1426.7	CA N^{ε}-Hexanoyllysine (170 Da)
HIRL	146–149	664.5/669.7	Octenal–His MA (126/131 Da)
HIRL	146–149	736.8	OUEA–His MA (198 Da)
HIRL	146–149	680.3/685.5	2-Octenoic acid–His MA (142/147 Da)
HIRL	146–149	752.4	2-Octenoic acid CA–His MA (214 Da)
HIRL	146–149	708.6/713.6	ONEA–His MA (170/175 Da)
HIRL	146–149	832.5/837.6	KODDE–His MA (294/299 Da)
HIRL	146–149	848.4/853.7	EKODE–His MA (310/315 Da)
HIRL	146–149	850.7/855.7	HKODE–His MA (312/317 Da)
HIRL	146–149	866.8/871.7	DHKODE–His MA (328/333 Da)
HIRL	146–149	884.5/889.6	His Unknown (346/351 Da)

$: bold and underlined residue was modified; *: Cys was *S*-carboxyamidomethylated with iodoacetamide; #: doubly charged peak; CA: carboxy analog, which is the corresponding cOCP partner of ωOCP.

Table S2. Modified Peptides Detected by LC-ESI-MS/MS from Tryptic Digest

Peptide sequence[$]	Position	Mass of modified	Assignment (Mass Shift)

		peptide	
ALPM**H**IR	142–148	993.4/998.4	HNE–His MA (156/161 Da)
ALPMo**H**IR	142–148	1009.3/1014.3	
ALPM**H**IR	142–148	1065.6	HODA–His MA (228 Da)
ALPMo**H**IR	142–148	1081.3	
LIVTQTMK	1–8	1087.4/1092.4	ONE–Lys 4-Ketoamide (154/159 Da)
LIVTQTMoK	1–8	1103.4/1108.4	
KIIAEK	70–75	855.3/860.5	
T**K**IPAVFK	76–83	1057.3/1062.6	
FD**K**ALK	136–141	875.3/880.4	
LIVTQTMK	1–8	1159.4	DODE–Lys 4-Ketoamide (226 Da)
LIVTQTMoK	1–8	1175.3	
KIIAEK	70–75	927.4	
T**K**IPAVFK	76–83	1129.3	
FD**K**ALK	136–141	947.2	
KIIAEK	70–75	837.4/842.4	ONE–Lys Pyrrolinone (136/141 Da)
KIIAEK	70–75	909.3	DODE–Lys Pyrrolinone (208 Da)
ALPMo**H**IR	142–148	979.3/984.4	Octenal–His MA (126/131 Da)
ALPMo**H**IR	142–148	1051.1	OUEA–His MA (198 Da)
ALPM**H**IR	142–148	979.3/984.4	2-Octenoic acid–His MA (142/147 Da)
ALPM**H**IR	142–148	1051.2	2-Octenoic acid CA–His MA (214 Da)
ALPMo**H**IR	142–148	1163.6/1168.5	EKODE–His (310/315 Da)

$: bold and underlined residue was modified; CA: carboxy analog, which is the corresponding cOCP partner of ωOCP.

Table S3. Modified Peptides Detected by LC-ESI-MS/MS from Chymotryptic Digest after Reduction

Peptide sequence[$]	Position	Mass of modified peptide	Assignment[@] (Mass Shift)
HIRL	146-149	696.3/701.4	HNE-His MA (158/163 Da)
SFNPTQLEEQC[*]**H**I	150-162	1760.4/1765.5	
HIRL	146-149	768.3	HODA-His MA (230 Da)
SFNPTQLEEQC[*]**H**I	150-162	1832.5	
VEEL**K**PTPEGDLEIL	43-57	1837.6/1842.9	ONE-Lys 4-Ketoamide (156/161 Da)
LQ**K**W	58-61	730.3/735.2	
KIPAVF	77-82	830.5/835.6	
KIDAL	83-87	715.2/720.1	
KIDALNEN**K**VL	83-93	1412.6/1417.5	
K**K**Y	100-102	594.2/599.3	
VRTPEVDDEALE**K**F	123-136	1803.7/1808.8	
D**K**AL	137-140	602.1/607.1	
VEEL**K**PTPEGDLEIL	43-57	1909.6	KODA-Lys 4-Ketoamide (228 Da)
LQ**K**W	58-61	802.3	
KIPAVF	77-82	902.3	
KIDAL	83-87	787.2	
KIDALNEN**K**VL	83-93	1484.6	
NEN**K**VL	88-93	944.2	
KKY	100-102	666.2	
K**K**Y	100-102	666.2	
VRTPEVDDEALE**K**F	123-136	1875.6	
D**K**AL	137-140	674.1	
VRTPEVDDEALE**K**F + AC[*]Q**C**L	123-136 + 118-122	1180.5/1182.6[#]	Cys-ONE-Lys Pyrrole Cross-link (118/123 Da)
VRTPEVDDEALE**K**F + **C**L	123-136 + 121-122	1001.3/1004.3[#]	
VRTPEVDDEALE**K**F + AC[*]Q**C**L	123-136 + 118-122	1216.4[#]	Cys-KODA-Lys Pyrrole Cross-link (190 Da)
VRTPEVDDEALE**K**F + **C**L	123-136 + 121-122	1036.2[#]	
LIVTQ + **H**IRL	1-5 + 146-149	1228.3/1233.5	His-ONE-Lys Pyrrole Cross-link (118/123 Da)
KGL + **H**IRL	8-10 + 146-149	972.2/977.5	
VEEL**K**PTPEGDLEIL + **H**IRL	43-57 + 146-149	1169.2/1172.0[#]	
KIDAL + **H**IRL	83-87 + 146-149	1214.4/1219.5	

KIDALNEN**K**VL + **H**IRL	83-93 + 146-149	956.7/959.1[#]	
KKY + **H**IRL	100-102 + 146-149	1093.3/1098.3	
K**K**Y + **H**IRL	100-102 + 146-149	1093.5/1098.4	
VRTPEVDDEALE**K**F + **H**IRL	123-136 + 146-149	1152.4/1154.7[#]	
D**K**AL + **H**IRL	137-140 + 146-149	1101.7/1106.5	
LIVTQ + **H**IRL	1-5 + 146-149	1300.4	
KGL + **H**IRL	8-10 + 146-149	1044.3	
VEEL**K**PTPEGDLEIL + **H**IRL	43-57 + 146-149	1205.7[#]	His-KODA-Lys Pyrrole Cross-link (190 Da)
KIDAL + **H**IRL	83-87 + 146-149	1286.6	
KIDALNEN**K**VL + **H**IRL	83-93 + 146-149	992.7[#]	
VRTPEVDDEALE**K**F + **H**IRL	123-136 + 146-149	1188.4[#]	
D**K**AL + **H**IRL	137-140 + 146-149	1173.8	
VEEL**K**PTPEGDLEIL	43-57	1851.7	CA N^{ε}-Hexanoyllysine (170 Da)
KIDALNEN**K**VL	83-93	1354.6/1359.4	N^{ε}-Hexanoyllysine (98/103 Da)
KIDALNEN**K**VL	83-93	1426.7	CA N^{ε}-Hexanoyllysine (170 Da)
HIRL	146-149	666.4/671.3	Octenal-His MA (128/133 Da)
HIRL	146-149	738.2	OUEA-His MA (200 Da)
HIRL	146-149	834.4/839.0	KODDE-His MA (296/301 Da)
HIRL	146-149	850.4/855.4	EKODE-His MA (312/317 Da)
HIRL	146-149	886.4/891.4	His Unknown (348/353 Da)
HIRL	146-149	680.7/685.6	2-Octenoic acid-His MA (142/147 Da)
HIRL	146-149	752.5	2-Octenoic acid CA-His MA (214 Da)
HIRL	146-149	710.5/715.6	ONEA-His MA (172/177 Da)
HIRL	146-149	782.5	ONEA CA-His MA (244 Da)

$: bold and underlined residue was modified; *: Cys was *S*-carboxyamidomethylated with iodoacetamide; @: reduced form if reducible; #: doubly charged peak; CA: carboxy analog, which is the corresponding cOCP partner of ωOCP.

Table S4. Modified Peptides Detected by LC-ESI-MS/MS from Tryptic Digest after Reduction

Peptide sequence[$]	Position	Mass of modified peptide	Assignment[@] (Mass Shift)
ALPM**H**IR	142-148	995.4/1000.4	HNE-His MA (158/163 Da)
ALPMo**H**IR	142-148	1011.4/1016.3	
ALPM**H**IR	142-148	1067.4	HODA-His MA (230 Da)
ALPMo**H**IR	142-148	1083.3	
LIVTQTMK	1-8	1089.3/1094.3	ONE-Lys 4-Ketoamide (156/161 Da)
LIVTQTMoK	1-8	1105.4/1110.2	
KIIAEK	70-75	857.2/862.4	
IIAE**K**TK	71-77	958.2/963.2	
T**K**IPAVFK	76-83	1059.4/1064.2	
FD**K**ALK	136-141	877.4/882.4	
LIVTQTMK	1-8	1161.4	KODA-Lys 4-Ketoamide (228 Da)
LIVTQTMoK	1-8	1177.5	
KIIAEK	70-75	929.3	
IIAE**K**TK	71-77	1030.4	
T**K**IPAVFK	76-83	1131.5	
FD**K**ALK	136-141	949.2	
ALPM**H**IR	142-148	979.4/984.6	2-Octenoic acid-His MA (142/147 Da)
ALPM**H**IR	142-148	1051.2	2-Octenoic acid CA-His MA (214 Da)
ALPM**H**IR	142-148	1149.5/1154.5	EKODE-His MA (312/317 Da)
ALPMo**H**IR	142-148	1165.3-1170.4	EKODE-His MA (312/317 Da)

$: bold and underlined residue was modified; @: reduced form if reducible; CA: carboxy analog, which is the corresponding cOCP partner of ωOCP.

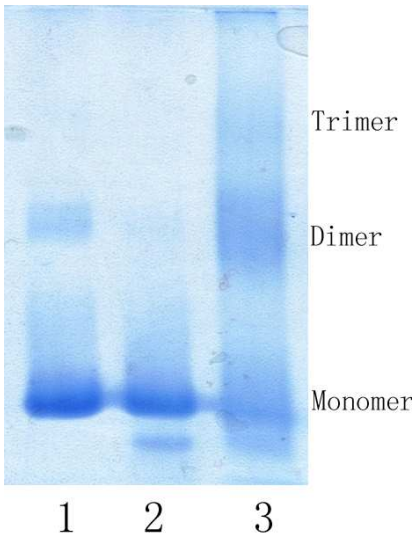

Figure S1. SDS–PAGE of fresh β-LG (lane 1), 0.1 mM β-LG incubated at pH 7.4 HEPES buffer at 37 °C for 3 d (lane 2) and 0.1 mM β-LG incubated with 5 mM LA, 1 mM Asc and 0.5 mM $FeSO_4 \bullet (NH_4)_2SO_4$ at pH 7.4 HEPES buffer at 37 °C for 3 d (lane 3).

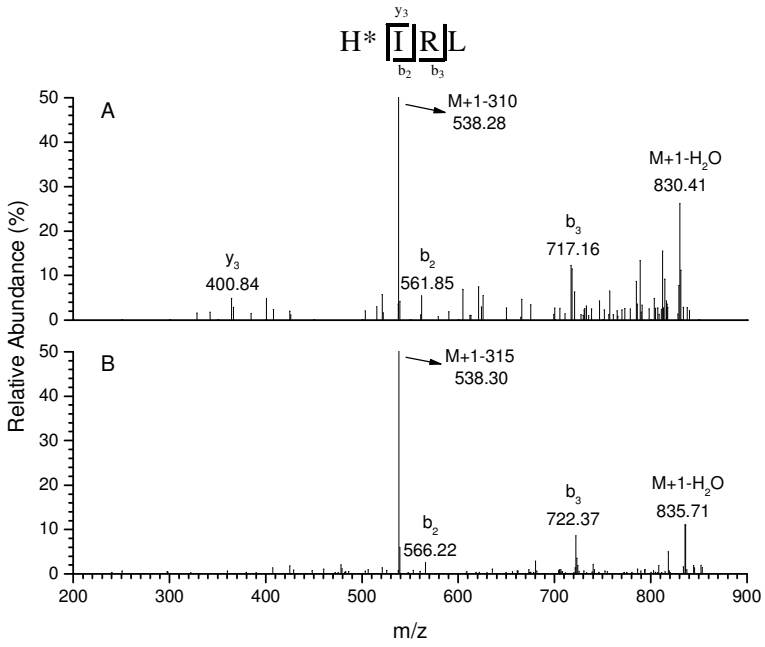

Figure S2. Tandem mass spectra of the modified HIRL at H146 by d_0-EKODE (A) and d_5-EKODE (B) through Michael addition.

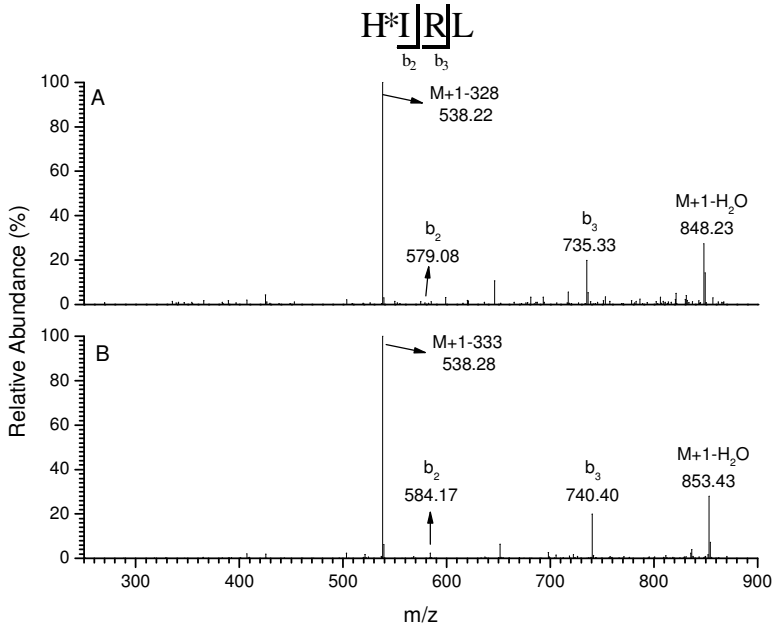

Figure S3. Tandem mass spectra of the modified HIRL at H146 by d_0-DHKODE (A) and d_5-DHKODE (B) through Michael addition.