Hypochlorous Acid Reacts with the N-Terminal Methionines of Proteins to Give Dehydromethionine, a Potential Biomarker for Neutrophil-Induced Oxidative Stress

Jennifer L. Beal, Steven B. Foster, and Michael T. Ashby*

Department of Chemistry and Biochemistry, University of Oklahoma, 620 Parrington Oval, Norman, OK 73019

Submitted to *Biochemistry* August 4, 2009

SUPPORTING INFORMATION

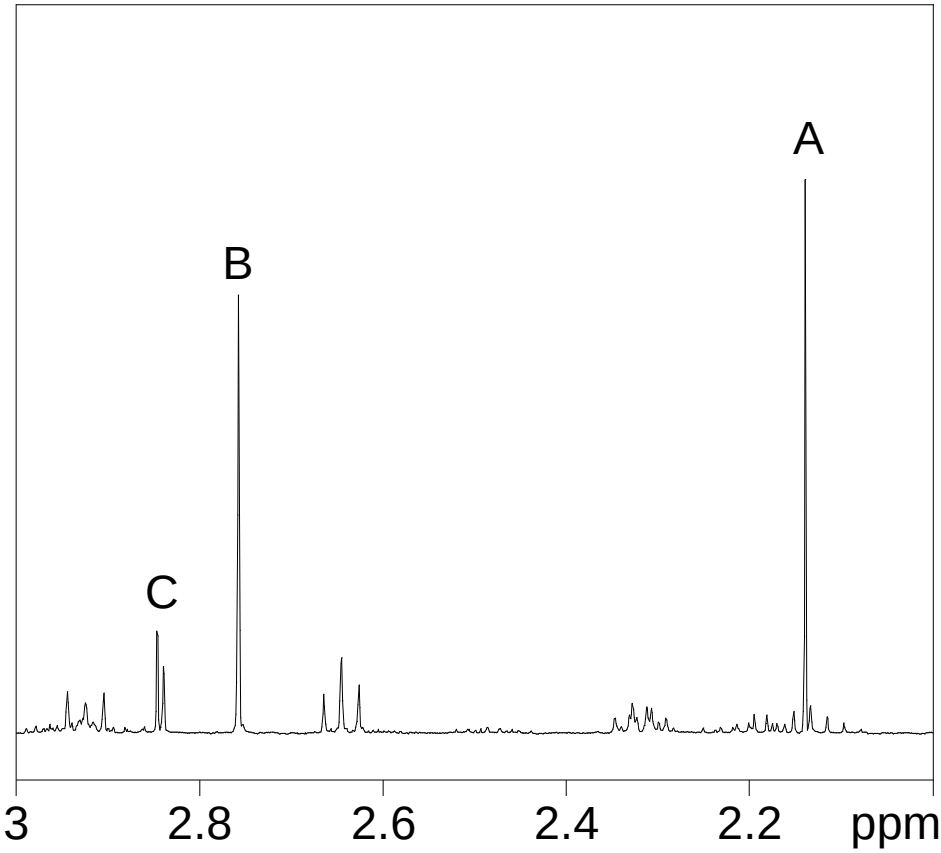

Figure S1. Representative ^{1}H NMR spectrum of the –S-CH_3 region of free methionine derivatives obtained from the reaction of 30 mM Met with 25 mM halogenating agent in 0.2 M phosphate buffer, pH=7.4-7.6. This spectrum was obtained from the oxidation of Met with HOCl at pH=7.5. The labeled resonances are S-CH_3 of methionine (A), methionine sulfoxide (B), and dehydromethionine (C). The methyl resonances labeled B and C were integrated to give the chemical yields that are reported in Table 2.

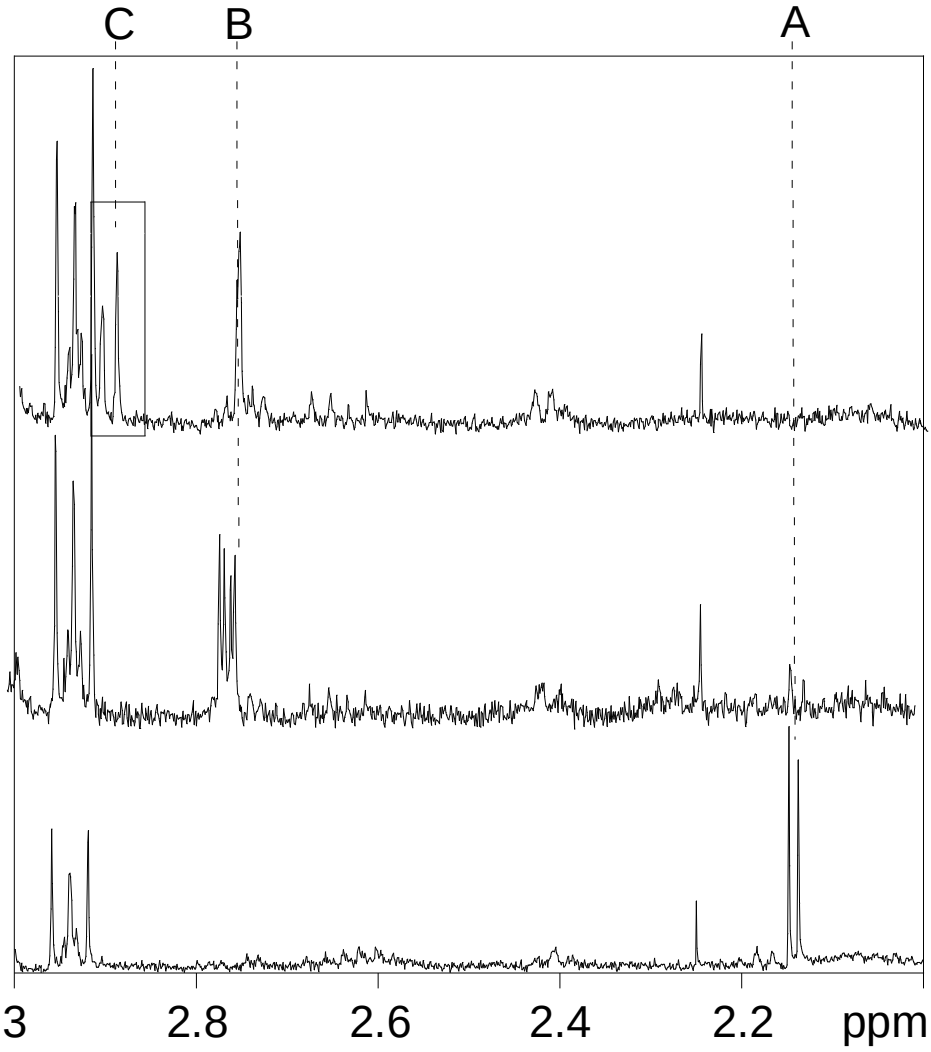

Figure S2. ^{1}H NMR spectra of antiflammin-1 (30 µM) in 0.1 M pD PBS (pH=7.4): native (bottom), oxidized with 10 % molar excess of H_2O_2 (middle), and oxidized with 10 % molar excess I_3^- (top). Note that the triplet at 2.9 ppm is due to the internal standard DSS. The labeled resonances are S-CH_3 of methionine (A), methionine sulfoxide (B), and dehydromethionine (C).

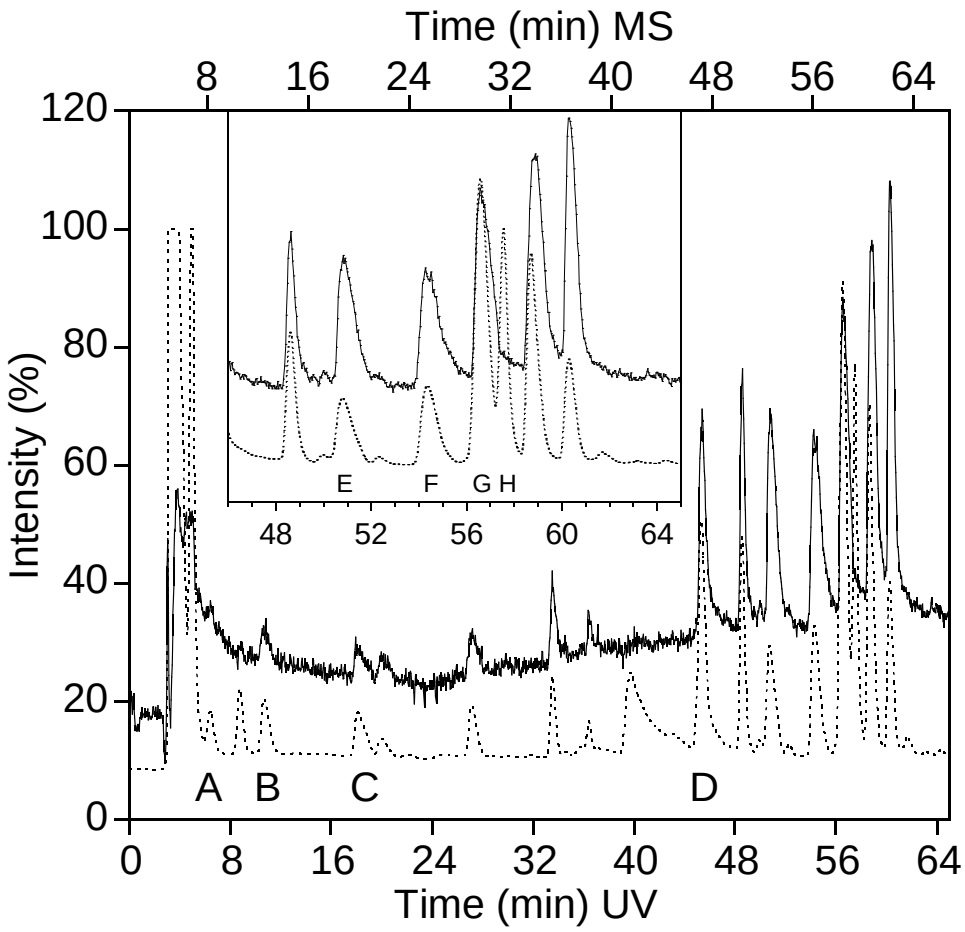

Figure S3. Representative MS (solid) and LC (dotted, λ = 205 nm) chromatograms of digested Ub. The scale of the MS chromatogram is shifted by 1.8 min to normalize the two chromatograms. The following peptides from the digested Ub have been assigned (based on observed m/z): A: Leu-Arg-Gly-Gly (73-76); B: Thr-Leu-Thr-Gly-Lys (7-11); C: Gln-Leu-Glu-Asp-Gly-Arg (49-54); D: Thr-Leu-Ser-Asp-Tyr-Asn-Ile-Gln-Lys (55-63); E: Leu-Ile-Phe-Ala-Gly-Lys (43-48); F: Met-Gln-Ile-Phe-Val-Lys (1-6); G: Glu-Ser-Thr-Leu-His-Leu-Val-Leu-Arg (64-72); H: Thr-Ile-Thr-Leu-Glu-Val-Glu-Pro-Ser-Asp-Thr-Ile-Glu-Asn-Val-Lys (12-27). A-G were identified by m/z of $(M+H)^{1+}$, and H was identified as $(M+H)^{2+}$. Unidentified peaks may be due to autodigestion of trypsin. **Inset:** expansion of the time between 45 and 65 minutes.

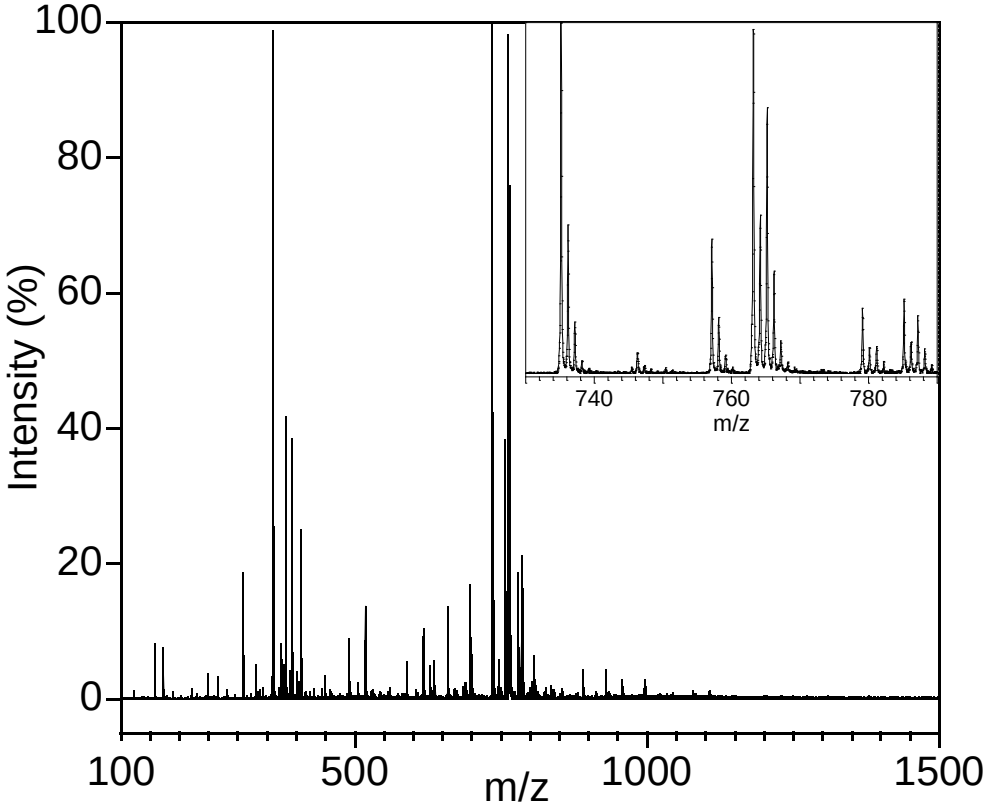

Figure S4. MS-ESI$^+$ of the DHM derivative of Ub1-6 (prepared in water, by addition of a small excess of I_3^-; pH after reaction was 3.4; ^{1}H NMR was used to characterize the product). The sample was diluted by MeOH and directly injected into the mass spectrometer. To minimize conversion of the DHM to MetO, the MS was obtained within 10 min of preparation. Dominant peaks were observed at 360.2 m/z, 735.2 m/z, and 763.2 m/z (attributed to the DHM-hexapeptide). **Insets:** expansion.

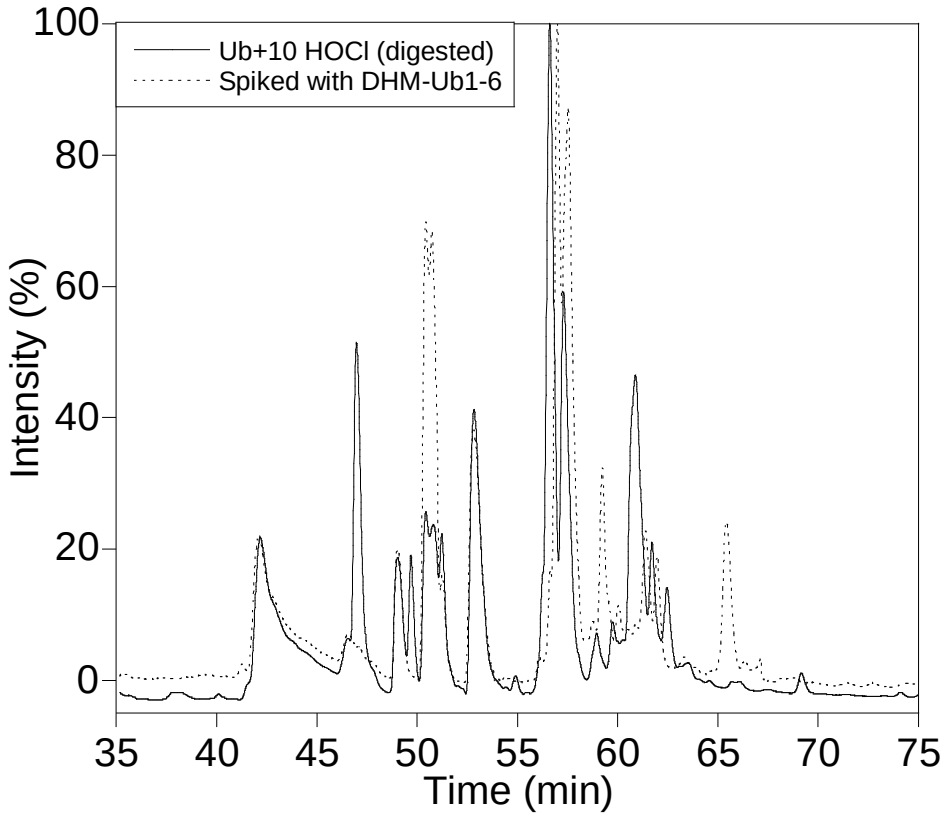

Figure S5. LC of Ub oxidized with 10 molar equivalents of HOCl and subsequently digested using trypsin (solid line). Same sample spiked (dotted line) with an authentic sample of the DHM derivative of Ub1-6 (prepared in 0.1M phosphate buffer by adding a 10 % molar excess of I_3^-; the product was characterized by ^{1}H NMR) in order to verify retention time. Note that the excess I_3^- from the DHM preparation reacts with a peptide in the digest (Thr-Leu-Ser-Asp-Tyr-Asn-Ile-Gln-Lys, residues 55-63 of U bar 46 min rt) to give the corresponding iodo- and diiodo Tyr derivatives (rt 59 and 66). The peak at rt = 62 is not part of the Ub digest, but a fragment of the trypsin autodigest.