**Supplementary Material**

# Inactivation of thiol-dependent enzymes by hypothiocyanous acid: role of sulfenyl thiocyanate and sulfenic acid intermediates.

Tessa J. Barrett, David I. Pattison, Stephen E. Leonard, Kate S. Carroll, Michael J. Davies and Clare L. Hawkins

**Supplementary Figure 1:** Treatment of CK with HOSCN results in generation of sulfenic acid formation at Cys-73. CK (25 μM) was treated with HOSCN (12.5 – 250 μM) for 5 min, prior to reductive alkylation, tryptic digestion and LC/MS/MS analysis. (a) No loss of the alkylated parent Cys-73 containing peptide of CK was observed (Cys-73, $[M+3H]^{3+}$, 1559.7.); (b) formation of the +138 product peptide corresponding to the addition of dimedone at the Cys-73 site of CK ($[M+3H]^{3+}$, 1586.4) was apparent. Peak areas are standardized to the area of the oxidant insensitive CK peptide SEEEYPDLSK ($[M+2H]^{2+}$, 598.5).

* Represents a significant increase ($p < 0.05$) in the concentration of each peptide of compared to control 1-way ANOVA with Dunnett's post-hoc test. Values are means ± S.E.M ($n = 6$).

**Supplementary Figure 2:** Fragmentation ion spectra of $[M+3H]^{3+}$ and $[M+138+3H]^{3+}$ peptides from tryptic digests of HOSCN-treated CK. Data shown in (a) are (left-hand side) an extracted ion chromatogram obtained from the MS/MS of the triply charged species *m/z* 1559.7 (carboxymethyl peptide), with the fragment ions specific for the Cys73 + 58 product and (right-hand side) the corresponding MS/MS spectrum. Fragment ions are doubly charged. Equivalent data are shown in (b) are (left-hand side) obtained from monitoring the specific fragment ions from the Cys73 + 138 product (*m/z* 1586.4). Data are representative of at least five independent experiments.

**Supplementary Figure 3:** Treatment of GAPDH with HOSCN results in generation of sulfenic acid formation at the Cys-244 position. GAPDH (25 μM) was treated with HOSCN (12.5 – 250 μM) for 5 min, prior to reductive alkylation, tryptic digestion and LC/MS/MS analysis. (a) No loss of the parent Cys-244 containing peptide of GAPDH was observed (Cys-244, $[M+2H]^{2+}$, 779.9); however (b) formation of the +138 product peptide corresponding to the addition of dimedone at the Cys-244 site of GAPDH ($[M+2H]^{2+}$, 819.9) was apparent. Peak areas are standardized to the area of the oxidant insensitive GAPDH peptide GAAQNIIPASTGAAK ($[M+2H]^{2+}$, 685.6).

* Represents a significant increase ($p < 0.05$) in the concentration of each peptide of compared to control 1-way ANOVA with Dunnett's post-hoc test. Values are means ± S.E.M ($n = 6$).

**Supplementary Figure 4:** Fragmentation ion spectra of $[M+2H]^{2+}$ and $[M+138+2H]^{2+}$ peptides (containing Cys-244) from tryptic digests of HOSCN-treated GAPDH. Data shown in (a) are (left-hand side) an extracted ion chromatogram obtained from the MS/MS of the doubly charged species *m/z* 779.9 (carboxymethyl peptide), with the fragment ions specific for the Cys244 + 58 species and (right-hand side) the corresponding MS/MS spectrum. Equivalent data shown in (b) are (left-hand side) obtained from monitoring the specific fragment ions from the Cys244 + 138 product, *m/z* 819.9. Data are representative of at least five independent experiments.

**Supplementary Table 1**

The $[M+H]^+$ for all Cys-containing peptides includes alkylation of the Cys residue, corresponding to a mass increase of +58 *m/z* relative to the native peptide. All identified peptides have a cross correlation score ≥ 1.

| Sequence | $[M+H]^+$ | Location |
|---|---|---|
| LNYK | 537.6 | 11-14 |
| SEEEYPDLSK | 1197.2 | 15 – 24 |
| HNNHMAK | 851.9 | 25 – 31 |
| VLTPDLYK | 949.1 | 32 – 39 |
| DKETPSGFTLDDVIQTGVDNPGHPFIMTVG**C**$^{73}$VAGDEESY TVFK | 4677.0 | 43 – 85 |
| DLFDPIIQDR | 1232.3 | 86 – 95 |
| HGGFKPTDK | 987.1 | 96 – 104 |
| HKTDLNHENLK | 1349.5 | 105 – 115 |
| TDLNHENLK | 1084.1 | 107 – 115 |
| TDLNHENLKGGDDLDPHYVLSSR | 2596.8 | 107 – 129 |
| GGDDLDPHYVLSSR | 1531.6 | 116 - 129 |
| GYTLPPH**C**$^{145}$SR | 1189.3 | 138 – 147 |
| RAVEK | 602.7 | 151 – 155 |
| LSVEALNSLTGEFK | 1508.7 | 156 – 169 |
| YYPLK | 683.8 | 172 – 176 |
| SMTEQEQQQLIDDHFLFDKPVSPLLLASGMAR | 3647.1 | 177 – 208 |
| DWPDAR | 759.8 | 209 – 214 |

| | | |
|---|---|---|
| GIWHNDNK | 984.1 | 215 – 222 |
| SFLVWVNEEDHLR | 1644.8 | 223 – 235 |
| VISMEK | 706.9 | 236 – 241 |
| RF**C**[253]VGLQK | 1009.2 | 251 - 258 |
| IEEIFK | 778.9 | 259 - 264 |
| AGHPFMWNEHLGYVLT**C**[282]PSNLGTGLR | 2930.3 | 266 – 291 |
| LAHLSK | 668.8 | 298 - 303 |
| FEEILTR | 908.0 | 307 – 313 |
| RGTGGVDTAAVGSVFDISNADR | 2166.3 | 319 - 340 |
| LGSSEVEQVQLVVDGVK | 1787.01 | 341 – 357 |
| LMVEMEKK | 1008.3 | 358 – 365 |
| KLEK | 517.6 | 364 – 367 |
| GQSIDDMIPAQK | 1303.5 | 369 – 380 |

**Supplementary Table 2**

The $[M+H]^+$ for all Cys-containing peptides includes alkylation of the Cys residue, corresponding to a mass increase of +58 *m/z* relative to the native peptide. All identified peptides have a cross correlation score ≥ 1.

| Sequence | $[M+H]^+$ | Location |
|---|---|---|
| VGVNGFGR | 805.9 | 3-10 |
| AITIFQER | 978.1 | 70 - 77 |
| AGAHLK | 596.7 | 105 - 110 |
| RVIISAPSADAPMFVMGVNHEK | 2370.8 | 115 - 136 |
| VIISAPSADAPMFVMGVNHEK | 2214.6 | 116 - 136 |
| IVSNAS**C**$^{149}$TTN**C**$^{153}$LAPLAK | 1823.0 | 143 - 159 |
| VIHDHFGIVEGLMTTVHAITATQK | 2620.0 | 160 - 183 |
| GAAQNIIPASTGAAK | 1370.5 | 198 - 212 |
| VIPELNGK | 870.0 | 217 - 224 |
| LTGMAFR | 795.9 | 225 - 231 |
| VPTPNVSVVDLT**C**$^{244}$R | 1558.8 | 232 - 245 |
| YDDIK | 653.7 | 252 - 256 |
| VVDLMVHMASK | 1230.5 | 321 - 331 |
| VVDLMVHMASKE | 1359.7 | 321 - 332 |

**Supplementary Table 3**

MS and HPLC features of the different CK active site containing peptides observed after HOSCN treatment on tryptic digestion.

Sequence: AGHPFMWNEHLGYVLTC$^{282}$PSNLGTGLR

| Assignment | $[M+2H]^{2+}$ | Retention Time (min) | EIC parameters |
|---|---|---|---|
| $Cys^{282}$ + 58 | 1465.6 | 25.5 | 913.0-915.0, 1074.0-1076.0, 1288.0-1290.0, 1319.0-1321.0, 1331.0-1333.0, 1607.0-1609.0, 1638.0-1640.0 |
| $Cys^{282}$ + 138 | 1505.6 | 28.0 | 913.0-915.0, 969.0-971.0, 1155.0-1157.0, 1440.0-1442.0, 1468.0-1470.0, 1540.0-1542.0, 1854.0-1856.0 |
| $Cys^{282}$ + 32 | 1452.6 | 25.9 | 913.0-915.0, 1418.0-1420.0, 1889.0-1990.0 |
| $Cys^{282}$ + 48 | 1460.6 | 23.9 | 502.0-504.0, 913.0-915.0, 1278.0-1280.0, 1373.0-1375.0, 1540.0-1542.0, 1639.0-1641.0, 1752.0-1754.0 |

**Supplementary Table 4**

MS and HPLC features of the different GAPDH active site containing peptides observed after HOSCN treatment on tryptic digestion.

Sequence: IVSNASC$^{149}$TTNC$^{153}$LAPLAK

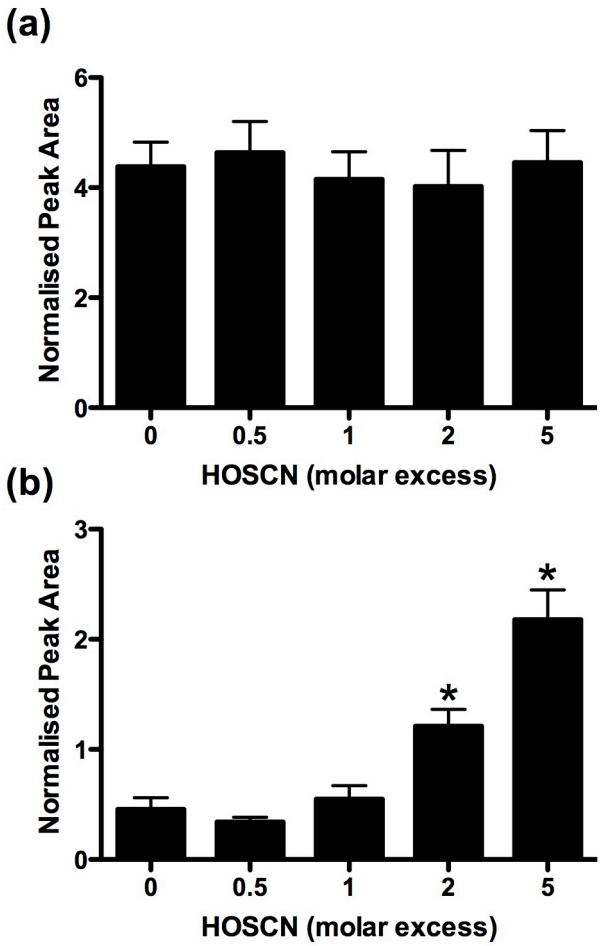
| Assignment | $[M+2H]^{2+}$ | Retention Time (min) | EIC parameters |
|---|---|---|---|
| $Cys^{149}$ + 58, $Cys^{153}$ + 58 | 912.0 | 18.1 | 427.0-429.0, 498.0-500.0, 772.0-774.0, 1088.0-1090.0, 1249.0-1251.0, 1336.0-1338.0, 1393.0-1395.0 |
| $Cys^{149}$ + 138, $Cys^{153}$ + 58 | 952.0 | 21.1 | 988.0-990.0, 1089.0-1091.0, 1015.0-1017.0 |
| $Cys^{149}$ + 32, $Cys^{153}$ + 58 | 899.0 | 17.0 | 706.0-708.0, 772.0-774.0, 807.0-809.0, 887.0-889.0, 907.0-909.0, 988.0-990.0, 1023.0-1025.0, 1088.0-1090.0 |
| $Cys^{149}$ + 48, $Cys^{153}$ + 58 | 907.0 | 15.9 | 722.0-724.0, 772.0-774.0, 823.0-825.0, 887.0-889.0, 924.0-926.0, 988.0-990.0, 1089.0-1091.0 |

**Supplementary Table 5**

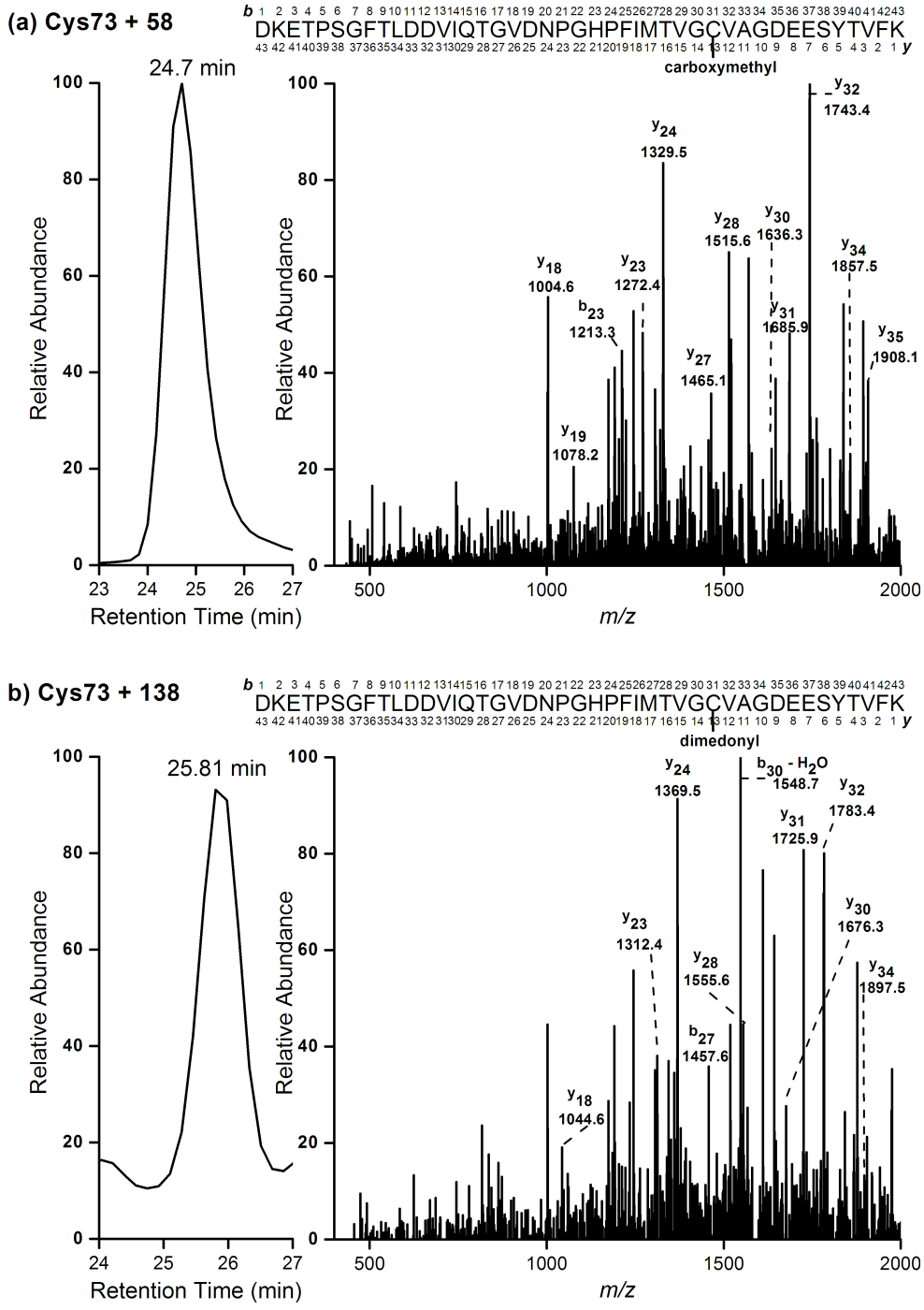
Parameters associated with CK and GAPDH identification from excised gel bands.

| **Identity** | **Matched Peptides** | **Sequence Coverage (%)** | **$M_r$** | **Swiss-Prot ID** |
|---|---|---|---|---|
| GAPDH | 11 | 47 | 35810 | P16858 |
| CK | 7 | 23 | 42714 | Q0447 |

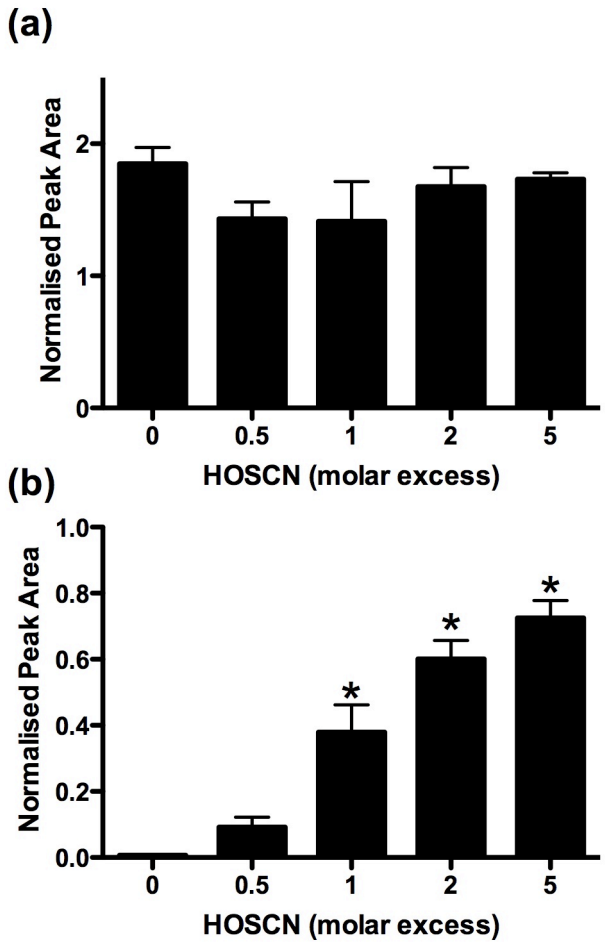
**Supplementary Figure 1**



**Supplementary Figure 2**



**Supplementary Figure 3**



**Supplementary Figure 4**