**Supporting Information for**

Serum electrolytes can promote hydroxyl radical-initiated biomolecular damage from inflammation

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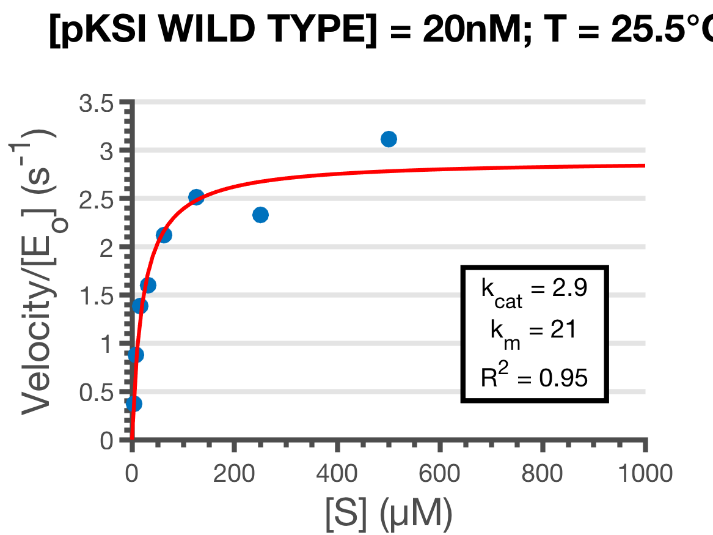
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**Fig. S1.** Example enzymatic saturation curve assay results for one replicate for KSI wild type with the substrate 5(10)-estrene-3,17-dione. Enzyme was diluted from an 8 µM solution containing serum electrolytes (100 mM NaCl, 60 μM NaBr and 20 mM NaHCO3) in 10 mM phosphate buffer at pH 7.4. Example is for non-γ-irradiated KSI wild type at 10 nM with 3.9 – 500 µM 5(10)-esterene-3,17-dione.

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**Fig. S2.** Effect of serum electrolytes on the •OH-mediated degradation of *N*-acetylated aromatic amino acids.Degradation of 50 μM each of 20 *N*-acetylated common amino acids vs. cumulative •OH produced at 4.4 μM/min using γ-radiolysis in 10 mM phosphate buffer at pH 7.4 with or without serum electrolytes (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3). Data for *N*-acetylated aromatic amino acids provided here. See Figs. S3-S6 for other *N*-acetylated amino acids. Error bars represent the standard error of quadruplicate experiments. Asterisk (\*) indicates area under the curve (AUC) difference between with and without serum electrolytes is ≥25% with ≤25% standard error difference**.**

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**Fig. S3.** Effect of serum electrolytes on the •OH-mediated degradation of *N*-acetylated non-polar aliphatic amino acids.Degradation of 50 μM each of 20 *N*-acetylated common amino acids vs. cumulative •OH produced at 4.4 μM/min using γ-radiolysis in 10 mM phosphate buffer at pH 7.4 with or without serum electrolytes (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3). Data for *N*-acetylated non-polar aliphatic amino acids provided here. See Fig. S2 and Figs. S4-S6 for other *N*-acetylated amino acids. *N*-Acetyl glycine analysis was challenging due to its short chromatographic retention time (Table S2). The apparent increase in *N*-acetyl valine concentration may derive from its formation from the degradation of *N*-acetyl isoleucine. Error bars represent the standard error of quadruplicate experiments. Asterisk (\*) indicates area under the curve (AUC) difference between with and without serum electrolytes is ≥25% with ≤25% standard error difference**.**

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**Fig. S4.** Effect of serum electrolytes on the •OH-mediated degradation of *N*-acetylated positively-charged amino acids. Degradation of 50 μM each of 20 *N*-acetylated common amino acids vs. cumulative •OH produced at 4.4 μM/min using γ-radiolysis in 10 mM phosphate buffer at pH 7.4 with or without serum electrolytes (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3). Data for *N*-acetylated positively-charged amino acids provided here. See Figs. S2, S3, S5, and S6 for other *N*-acetylated amino acids. Error bars represent the standard error of quadruplicate experiments. Asterisk (\*) indicates area under the curve (AUC) difference between with and without serum electrolytes is ≥25% with ≤25% standard error difference**.**

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**Fig. S5.** Effect of serum electrolytes on the •OH-mediated degradation of *N*-acetylated negatively-charged amino acids.Degradation of 50 μM each of 20 *N*-acetylated common amino acids vs. cumulative •OH produced at 4.4 μM/min using γ-radiolysis in 10 mM phosphate buffer at pH 7.4 with or without serum electrolytes (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3). Data for *N*-acetylated negatively-charged amino acids provided here. See Figs. S2-S4 and Fig. S6 for other *N*-acetylated amino acids. *N*-Acetyl aspartic acid analysis was challenging due to its short chromatographic retention time (Table S2). Error bars represent the standard error of quadruplicate experiments. Asterisk (\*) indicates area under the curve (AUC) difference between with and without serum electrolytes is ≥25% with ≤25% standard error difference**.**

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**Fig. S6.** Effect of serum electrolytes on the •OH-mediated degradation of *N*-acetylated polar aliphatic amino acids.Degradation of 50 μM each of 20 *N*-acetylated common amino acids vs. cumulative •OH produced at 4.4 μM/min using γ-radiolysis in 10 mM phosphate buffer at pH 7.4 with or without serum electrolytes (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3). Data for *N*-acetylated polar aliphatic amino acids provided here. See Figs. S2-S5 for other *N*-acetylated amino acids. *N*-Acetylated serine, threonine, asparagine and glutamine analyses were challenging due to their short chromatographic retention times (Table S2). Error bars represent the standard error of quadruplicate experiments. Asterisk (\*) indicates area under the curve (AUC) difference between with and without serum electrolytes is ≥25% with ≤25% standard error difference**.**

**Table S1:** **Size and numbers of amino acid residues for the proteins**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Protein** | **Size (kDa)** | **Number of Amino Acids** | | | | | | |
| **Total** | **Tyr** | **Met** | **Ala** | **Trp** | **Cys** | **His** | | **Pro** |
| Human Serum Albumin | 66.5 | 585 | 18 | 6 | 62 | 1 | 35 | 16 | | 24 |
| Catalase\* | 250 | 2108 | 84 | 44 | 152 | 24 | 4 | 22 | | 37 |
| Carbonic anhydrase | 30 | 239 | 9 | 4 | 16 | 2 | 4 | 18 | | 9 |
| KSI (Wild Type) | 14.5 | 131 | 4 | 7 | 12 | 2 | 3 | 3 | | 8 |
| KSI (Y32F/Y57F/Y119F) | 14.5 | 131 | 1 | 7 | 12 | 2 | 3 | 3 | | 8 |
| \*Catalase is a tetramer; the values provided here are for the tetramer | | | | | | | | | | |

**Table S2:** **LC-MS/MS details for quantification of liberated amino acids, free *N*-acetyl amino acids and the internal standard.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Analyte** | **Retention Time, min** | **Parent ion m/z (M-1)** | **Daughter ion** | **Fragmentor Voltage, V** | **Collision Energy, V** | **Cell Accelerator Voltage, V** |
| AQC-Alanine | 5.8 | 258.1 | 88.1 | 84 | 5 | 7 |
| AQC-Methionine | 6.3 | 318.1 | 148.1 | 84 | 5 | 7 |
| AQC-Histidine | 5.6 | 324.1 | 154.1 | 84 | 5 | 7 |
| AQC-Tyrosine | 6.1 | 350.1 | 180.1 | 84 | 4 | 7 |
| AQC-Tryptophan | 6.5 | 373.1 | 203.1 | 84 | 5 | 7 |
| AQC-γ-Aminobutyric acid | 5.8 | 272.1 | 102.1 | 84 | 5 | 7 |
| \**N*-Acetyl Glycine | 1.06 | 118.1 | 76.1 | 65 | 5 | 7 |
| *N*-Acetyl Alanine | 1.6 | 130.1 | 88.0 | 84 | 5 | 7 |
| *N*-Acetyl Serine | 1.03 | 146.1 | 116.1 | 84 | 5 | 7 |
| \**N*-Acetyl Proline | 5.3 | 158.1 | 70.1 | 65 | 5 | 7 |
| *N*-Acetyl Valine | 6.0 | 158.1 | 116.1 | 84 | 9 | 7 |
| *N*-Acetyl Threonine | 1.3 | 160.1 | 74.1 | 84 | 9 | 7 |
| \**N*-Acetyl Cysteine | 2.3 | 164.0 | 122.0 | 60 | 5 | 7 |
| *N*-Acetyl Leucine | 6.6 | 172.1 | 130.1 | 84 | 9 | 7 |
| \**N*-Acetyl Isoleucine | 6.5 | 174.1 | 86.1 | 55 | 13 | 7 |
| *N*-Acetyl Asparagine | 0.94 | 173.1 | 155.0 | 84 | 9 | 7 |
| *N*-Acetyl Aspartic Acid | 0.93 | 174.1 | 88.0 | 84 | 5 | 7 |
| *N*-Acetyl Glutamic Acid | 1.1 | 188.1 | 128.0 | 84 | 9 | 7 |
| \**N*-Acetyl Glutamine | 0.89 | 189.1 | 130.0 | 80 | 9 | 7 |
| *N*-Acetyl Lysine | 0.85 | 187.1 | 145.2 | 84 | 9 | 7 |
| *N*-Acetyl Methionine | 6.1 | 190.1 | 148.1 | 84 | 9 | 7 |
| *N*-Acetyl Histidine | 0.92 | 196.1 | 154.1 | 84 | 9 | 7 |
| \**N*-Acetyl Arginine | 1.1 | 217.1 | 70.1 | 115 | 20 | 7 |
| *N*-Acetyl Tyrosine | 6.3 | 222.1 | 180.1 | 138 | 4 | 7 |
| *N*-Acetyl Tryptophan | 6.9 | 245.1 | 203.1 | 138 | 5 | 7 |
| *N*–Acetyl Phenylalanine | 6.8 | 206.1 | 164.0 | 84 | 1 | 7 |
|  | | | | | | |
| \*Indicates positive ion mode | | | | | | |

**Table S3: Area Under the Curve (AUC) analysis results for *N*-acetylated amino acids**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***N*-Acetyl Amino Acid**  **R-Groups** | **No Serum Electrolytes**  **AUC** | **No Serum Electrolytes**  **Standard Deviation** | **Serum Electrolytes**  **AUC** | **Serum Electrolytes**  **Standard Deviation** | **AUC Difference (%)** | **AUC Standard Error Difference (%)** |
| Glycine | 1400 | 530 | 1700 | 400 | 18% | 66% |
| Alanine | 1500 | 24 | 1300 | 40 | 13% | 6% |
| Serine | 1200 | 46 | 930 | 25 | 20% | 6% |
| Proline\* | 1000 | 49 | 670 | 43 | 32% | 5% |
| Valine | 2500 | 130 | 2200 | 170 | 11% | 19% |
| Threonine | 1100 | 620 | 890 | 210 | 22% | 65% |
| Cysteine\* | 120 | 3.6 | 39 | 5.1 | 66% | 2% |
| Leucine | 1300 | 42 | 1100 | 46 | 14% | 9% |
| Asparagine | 1200 | 62 | 1300 | 380 | 8% | 100% |
| Aspartic Acid | 1600 | 61 | 1800 | 190 | 13% | 24% |
| Isoleucine | 1200 | 57 | 1300 | 80 | 9% | 22% |
| Lysine | 1300 | 82 | 1100 | 110 | 16% | 17% |
| Glutamic Acid | 1400 | 55 | 1300 | 59 | 1% | 230% |
| Glutamine | 1200 | 90 | 1100 | 270 | 13% | 46% |
| Methionine\* | 570 | 25 | 430 | 34 | 25% | 7% |
| Histidine\* | 640 | 43 | 440 | 34 | 31% | 7% |
| Arginine | 940 | 100 | 760 | 58 | 19% | 16% |
| Tyrosine\* | 1200 | 200 | 590 | 41 | 52% | 8% |
| Tryptophan\* | 760 | 40 | 550 | 32 | 27% | 6% |
| Phenylalanine | 640 | 40 | 530 | 23 | 17% | 11% |

\**N*-acetyl amino acid features 1) a ≥25% difference between the means for the area under the curve (AUC; for *N*-acetyl amino acid concentration vs. •OH production) for the serum electrolytes and no serum electrolytes conditions and 2) a ≤25% standard error difference for these AUCs. See the discussion under the statistical analysis section of the methods.

**Table S4: Area Under Curve (AUC) analysis results for amino acids in proteins**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Amino Acid Residues** | **No Serum Electrolytes**  **AUC** | **No Serum Electrolytes**  **Standard Deviation** | **Serum Electrolytes**  **AUC** | **Serum Electrolytes**  **Standard Deviation** | **AUC Difference (%)** | **AUC Standard Error Difference (%)** |
| *Catalase* |  |  |  |  |  |  |
| \*Tyrosine | 110000 | 7200 | 23000 | 1300 | 79% | 3% |
| \*Tryptophan | 72000 | 5300 | 21000 | 1000 | 71% | 4% |
| Methionine | 48000 | 4400 | 60000 | 14000 | 26% | 39% |
| Alanine | 11000 | 28000 | 100000 | 140000 | 3% | 1700% |
| Histidine | 69000 | 24000 | 58000 | 58000 | 15% | 200% |
| *Human serum albumin* | |  |  |  |  |  |
| \*Tyrosine | 140000 | 3400 | 76000 | 72 | 44% | 2% |
| Tryptophan | 96000 | 6700 | 82000 | 7500 | 15% | 24% |
| \*Methionine | 34000 | 4800 | 88000 | 11000 | 160% | 8% |
| Alanine | 160000 | 11000 | 150000 | 190000 | 7% | 600% |
| Histidine | 110000 | 26000 | 120000 | 150000 | 9% | 500% |
| *Carbonic Anhydrase* |  |  |  |  |  |  |
| Tyrosine | 69000 | 23000 | 74000 | 28000 | 8% | 330% |
| Tryptophan | 45000 | 12000 | 48000 | 14000 | 7% | 280% |
| Methionine | 67000 | 27000 | 81000 | 32000 | 21% | 150% |
| Alanine | 140000 | 22000 | 160000 | 23000 | 10% | 110% |
| \*Histidine | 83000 | 11000 | 51000 | 5200 | 39% | 20% |
| *KSI (Wild Type)* |  |  |  |  |  |  |
| \*Tyrosine | 23000 | 3500 | 14000 | 2900 | 39% | 21% |
| \*Tryptophan | 15000 | 1800 | 7500 | 2100 | 50% | 13% |
| Methionine | 19000 | 3800 | 13000 | 5000 | 33% | 35% |
| Alanine | 26000 | 1100 | 23000 | 3500 | 15% | 15% |
| Histidine | 18000 | 4600 | 23000 | 20000 | 29% | 190% |

\*Amino acid features 1) a ≥25% difference between the means for the area under the curve (AUC; for amino acid vs. •OH production) for the serum electrolytes and no serum electrolytes conditions and 2) a ≤25% standard error difference for these AUCs. See the discussion under the statistical analysis section of the Methods.

**Table S5: Area Under Curve (AUC) analysis results for enzymatic activity**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Enzyme** | **No Serum Electrolytes**  **AUC** | **No Serum Electrolytes**  **Standard Deviation** | **Serum Electrolytes**  **AUC** | **Serum Electrolytes**  **Standard Deviation** | **AUC Difference (%)** | **AUC Standard Error Difference (%)** |
| \*Catalase | 10000 | 410 | 1500 | 320 | 85% | 2% |
| \*Carbonic Anhydrase | 3000 | 320 | 4500 | 640 | 53% | 23% |
| \*KSI (Wild Type) | 4300 | 2.6 | 3200 | 1.3 | 27% | 0.1% |

\*Enzymatic activity features 1) a ≥25% difference between the means for the area under the curve (AUC; for activity vs. •OH production) for the serum electrolytes and no serum electrolytes conditions and 2) a ≤25% standard error difference for these AUCs. See the discussion under the statistical analysis section of the Methods.

**Table S6:** **Losses of** **tyrosine and activity for KSI wild type and Y32F/Y57F/Y19F after exposure to 66 µM •OH**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Matrix** | **Tyrosine Concentration Loss (Conc/Conco, %)** | | **Enzymatic Activity Loss (Activity/Activityo, %)** | |
| KSI  (Wild Type) | KSI (Y32F/Y57F/Y119F) | KSI  (Wild Type) | KSI (Y32F/Y57F/Y119F) |
| No serum electrolytes (10 mM  phosphate - pH 7.4) | 97 ± 12\* | 78 ± 3 | 47 ± 3 | 34 ± 0.7 |
| Ionic strength control  (57 mM phosphate – pH 7.4) | 95 ± 14\* | 91 ± 2 | 37 ± 2 | 21 ± 0.6 |
| Chloride and bromide  (100 mM NaCl, 60 μM NaBr, 10  mM phosphate – pH 7.4) | 90 ± 4 | - | 30 ± 10 | 27 ± 3 |
| Chloride only  (100 mM NaCl, 10 mM phosphate  – pH 7.4) | - | - | 34 ± 0.1 | 26 ± 0.6 |
| Serum electrolytes  (100 mM NaCl, 60 μM NaBr, 20 mM NaHCO3, 10 mM phosphate – pH 7.4) | 73 ± 18 | 55 ± 4 | 22 ± 1 | 7 ± 1 |
| Carbonate only  (20 mM NaHCO3, 10 mM  phosphate – pH 7.4) | 78 ± 5 | - | 21 ± 0.2 | 9 ± 2 |
| Error terms for tyrosine concentration loss are standard error for n = 5 (Wild Type) or 3 (Y32F/Y57F/Y119F), except for asterisk (\*), where error terms are ranges for experimental duplicates. Error terms for the enzymatic activity loss are ranges for experimental duplicates. | | | | |

**Table S7: Enzymatic kinetic constants for KSI wild type and Y32F/Y57F/Y19F**

|  |  |  |  |
| --- | --- | --- | --- |
| **Protein** | **Michaelis Menten constant – *K****M* **(µM)** | **Catalytic reaction constant – *kcat* (s-1)** | ***kcat* / *K****M*  **(µM-1 s-1)** |
| KSI (Wild Type) | 20 ± 3 | 2.7 ± 0.5 | 0.138 ± 0.005 |
| KSI (Wild Type) after 66 µM \*OH a | 53 ± 10 | 1.3 ± 0.01 | 0.024 ± 0.004 |
| KSI (Y32F/Y57F/Y19F) b | 29 ± 3 | 3.5 ± 0.5 | 0.124 ± 0.02 |
| a Exposure in the presence of serum electrolytes  b Error terms are the standard error for n = 5 experiments. Otherwise the error term is a range for n = 2 experiments. | | | |