Electronic Supplementary Information

Antioxidant Promotion of Tyrosine Nitration in the Presence of Copper(II)

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SI-1: Calibration curve for Ang I+O

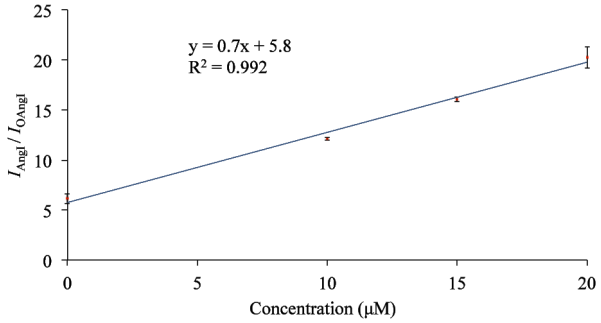

Figure SI-1. Standard addition calibration for Ang I+O. The intensity ratio obtained from mass spectra of Ang I and Ang I+O is plotted as a function of the concentration Ang I spike.

The standard addition method was used for calibration of Ang I+O. Ang I (0.2 mM) was incubated with nitrite (1 mM), ascorbic acid (1 mM) and $CuCl_2$ (0.025 mM) for 30 min under 25 °C to generate Ang I+O. The reaction product was quickly frozen and stored under −20 °C to stop oxidation or nitration for the standard addition calibration that was indeed immediately carried out after the 30 min reaction. 50 µl reaction product was mixed with 20 µl, 30 µl or 40 µl 0.25 mM Ang I and then diluted in ESI buffer to a final volume of 500 µl for MS analysis. The intensity ratio between Ang I (I_{AngI}) and Ang I+O (I_{OAngI}) from mass spectra was plotted against the added concentration of Ang I, as shown in figure SI-1. A good linearity was obtained, indicating that the concentration ratio between Ang I and Ang I+O is proposal to their intensity ratio on the mass spectra:

$$\frac{I_{\mathrm{AngI}}}{I_{\mathrm{OAngI}}} = a\frac{C_{\mathrm{AngI}}}{C_{\mathrm{OAngI}}} \quad (1)$$

Where C_{AngI} is the concentration of Ang I, including the remanent Ang I after 30 min of reaction ($C_{\mathrm{AngI}}(0)$) and the added Ang I (ΔC_{AngI}), and C_{OAngI} is the concentration of Ang I+O generated after 30 min of reaction ($C_{\mathrm{OAngI}}(0)$). Therefore, equation (1) can be written as:

$$\frac{I_{\mathrm{AngI}}}{I_{\mathrm{OAngI}}} = a\frac{\left(C_{\mathrm{AngI}}(0)+\Delta C_{\mathrm{AngI}}\right)}{C_{\mathrm{OAngI}}(0)} = \frac{a}{C_{\mathrm{OAngI}}(0)}\Delta C_{\mathrm{AngI}} + \frac{a}{C_{\mathrm{OAngI}}(0)}C_{\mathrm{AngI}}(0) \quad (2)$$

Considering figure SI-1, $C_{\mathrm{AngI}}(0)$ = 5.8/0.7 = 8 µM. Since only the Ang I+O and Ang I were observed on the mass spectra after 30 min of reaction, we assume that the rest

of the Ang I was all converted as Ang I+O. Therefore, $C_{OAngI}(0)$ = 12 μM, and then, a = 8.

This result shows that the Ang I+O is less efficient in the generation of triple protonated ions. In the sequence of Ang I, DRVYIHPFHL, 3 amino acid residues can be easily protonated in the strong acidic solution, including the 2nd amino acid R, the 6th amino acid H and the 9th amino acid H. In the Ang I+O, one histidine is oxidized and the oxidized histidine shows poorer affinity to proton.

SI-2: Angiotensin I oxidation by copper(II), oxygen, ascorbic acid and nitrite after 30 min of reaction

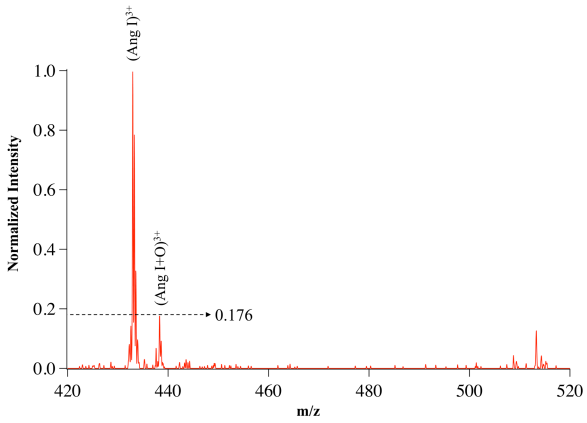

Figure SI-2. Mass spectrum of reaction products by incubating angiotensin I (Ang I, 0.25 mM) with $CuCl_2$ (0.025 mM), ascorbic acid (AA, 1 mM) and $NaNO_2$ (1 mM) in NH_4HCO_3 buffer (pH = 6) for 30 min under 37 °C and persistent shaking. The products were diluted by 10 times in the ESI buffer and infused at a flow rate of 10 µl/min into ESI-MS under ionization voltage of 3.7 kV.

As shown in the figure SI-2, only Ang I and Ang I+O can be detected by MS after 30 min of reaction by incubating Ang I (0.25 mM) with ascorbic acid (1 mM), NO_2^- (1 mM) and $CuCl_2$ (0.025 mM) at 37 °C under persistent shaking. The Ang I+O ratio can be calculated as $8\times0.176/(1 + 8\times0.176 + 0) = 0.585$, which is same as the Ang I+O ratio after 18 hours of reaction as shown in figure 3.