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SUPPORTING INFORMATION

Thiyl Radicals Are Co-Products of Dinitrosyl Iron Complex (DNIC) Formation

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Content:

EXPERIMENTAL SECTION

Materials and Methods

The reagents L-glutathione (reduced) (≥ 98.0%), L-cysteine (≥ 98.0%) and iron(II) sulfate heptahydrate (> 99.0%) were purchased from Sigma Aldrich. Solutions at pH 7.4 were prepared with HEPES buffer (≥ 99.5%).

Nitric oxide gas purification and solution preparation.

Nitric oxide gas (99.5%) from the tank (PraxAir) was passed through a stainless-steel column containing sodium hydroxide pellets to remove NO_2 and N_2O_3. The sodium hydroxide pellets were crushed and dried under vacuum before being packed into the column. In order to obtain solutions containing the desired NO concentration, the buffered solutions were deoxygenated in Schlenk flasks using the freeze-pump-thaw method. Then, NO gas was introduced to the headspace of the Schlenk flask on a vacuum line. The samples were allowed to equilibrate for a minimum of 40 min. The total pressure of the system was measured using a mercury manometer. The NO concentration was calculated by the partition coefficient between the gas and the liquid phases (K_H = 1.86 mM/atm at 298 K).[1,2]

Stopped-flow measurements.

Kinetics studies were performed in an upgraded Applied Photophysics model SX-18 MV stopped-flow spectrophotometer with a photomultiplier tube (PMT) as the detector. Gas-tight syringes with on-off valves were used to transfer solutions from Schlenk flasks to the stopped-flow instrument. The transfer syringes were purged several times with argon prior to insertion into the sample flasks. The reservoir syringes on the stopped-flow were flushed with several volumes of the deoxygenated buffer solution before the introduction of the sample solution. The temperature control was performed by a thermostatic, circulating water bath. The symmetrical mixing operation mode was used in all experiments. In general, one of the syringes was loaded with NO saturated HEPES buffer (200 mM) solution at pH 7.4 ([NO] = 1.86 mM, 1 atm and 298 K) while the second syringe was loaded with degassed HEPES buffer (200 mM)

solution at pH 7.4 containing ferrous sulfate ([Fe(II)] = 0.18 mM) and different concentrations of thiols.

EPR Spectroscopy.

The EPR spectra under continuous flow were recorded at room temperature (25 °C) on a Bruker EMX spectrometer operating at 9.71 GHz equipped with a Bruker ER4117 D-MTV dielectric mixing resonator with a 9-mm distance between the mixing cell and the resonator center (Fig. 1S). Two 10-ml gas-tight syringes were mounted on a syringe infusion pump (Harvard apparatus pump 22). In general, one of the syringes was loaded with NO saturated HEPES buffer (200 mM) solution at pH 7.4 ([NO] = 1.86 mM, 1 atm and 298 K) while the second syringe was loaded with degassed HEPES buffer (200 mM) solution at pH 7.4 containing ferrous sulfate ([Fe(II)] = 0.18 mM) and thiol (CysSH or GSH at 20mM). Spectra were recorded 140 ms after mixing at continuous flow of 0.5 mL/min. The magnetic field was calibrated with tempol.

In the spin trapping experiments, the EPR spectra were at room temperature on a Bruker ER 200 D-SRC that was upgraded to an EMX instrument equipped with a standard cavity. The experiments were performed at room temperature using a flat cell. The EPR instrument operated at 9.70 GHz microwave frequency, 120 G range, 1 G amplitude modulation, 100 kHz frequency modulation, 81.9 ms time constant, microwave power of 20 mW and 2 scans.

Figure S1. EPR spectrometer with mixing cell resonator for continuous flow measurements. (Photograph taken by coauthor D. R. Truzzi)

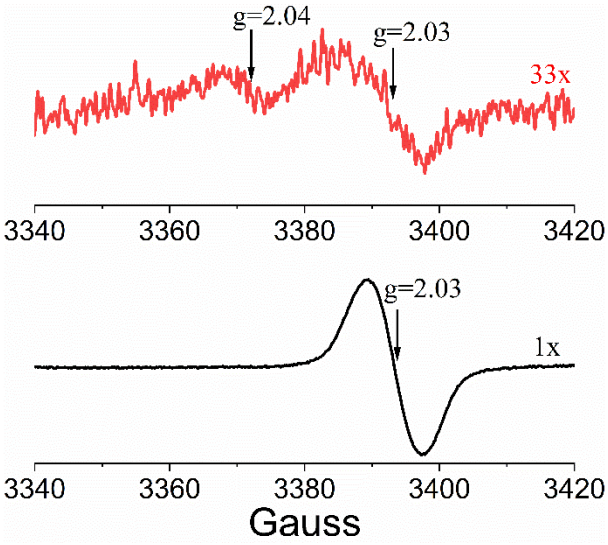

Figure S2 Temporal EPR spectra recorded using a flow cell mixer to prepare reaction solutions with final concentrations [Fe] = 0.09 mM, [NO] = 0.93 mM and [GSH] = 10 mM in pH 7.4 HEPES buffer (200 mM). *Upper:* EPR spectrum acquired 140 ms after mixing solutions at continuous flow of 0.5 ml/min. *Bottom*: EPR spectrum acquired 5s after stopping the solution flow. Instrumental conditions: microwave power, 2 mW; time constant, 81.9 ms; scan rate, 0.6 G/s; modulation amplitude, 5 G.

References

1 Lancaster, J. R. Nitroxidative, Nitrosative, and Nitrative Stress: Kinetic Predictions of Reactive Nitrogen Species Chemistry Under Biological Conditions. *Chem. Res. Toxicol.* **2006**, *19*, 1160–1174.

2 Young, C. L. (ed.) *Oxides of nitrogen*, 1st ed, Solubility data series, Pergamon Press, Oxford [Oxfordshire]; New York, 1981.