Electrophilic Sulfhydration of 8-nitro-cGMP involves sulfane sulfur.

Vida Terzić, Dominique Padovani, Véronique Balland, Isabelle Artaud and Erwan Galardon*

Physical Measurements.

Dioxygen concentration was monitored at 37°C with an Oroboros instrument. Oxygen consumption rates (JO2) were obtained directly from the Datlab 4 software. UV-Visible spectra were obtained on a Cary 300 Scan at 37°C. HPLC-MS (High Performance Liquid Chromatography coupled to Mass Spectrometry) or –HRMS (High Resolution Mass Spectrometry) spectra were recorded on a Thermo-Finnigan Surveyor equipped with a Satisfaction RP18 AB 3µm (150 x 2 mm) coupled to an ESI LCQ Advantage or with a Thermo-Scientific Accela-LC coupled to an Exactive orbitrap spectrometer using acetonitrile / aqueous 0.1% formic acid gradients. EPR spectra were obtained on an ALEXIS spectrometer (microwave frequency 9.45 GHz, modulation frequency 100 kHz, modulation amplitude 1 mT, microwave power 1 mW). Cyclic voltammetry was performed at 20°C using a platinum-wire auxiliary electrode, and a Ag/Ag/Cl/KCl reference electrode. The working electrode was a glassy carbon electrode and measurements were carried out in 0.1 M phosphate buffer at pH 7.4 carefully degassed by the bubbling of nitrogen before each measurement.

Materials.

Reagents were purchased from Aldrich or Alfa-Aesar and used as received. Hydrogen sulfide solutions were prepared by adding anhydrous sodium hydrosulfide (Alfa-Aesar) to degassed buffer containing 200 µM DTPA. 8-NO2-Gua was synthesized according to the published procedure1 and 8-NO2-cGMP was a gift from Pr. T. Akaike and Pr. T. Sawa. Authentic samples of 8-NH2-Gua and 8-SH-Gua were prepared as HPLC standards following published methods.2-3 The 1H NMR spectrum of 8-NH2-Gua is in agreement with the published data.4 8-SH-Gua: 1H NMR (δ, DMSO-d6, 250 MHz): 12.95 (s, 1H, SH), 11.06 (s, 1H, NH), 6.58 (br, 2H, NH2), 6.30 (d, 1H, J = 5.3Hz, C1'H), 5.26 (br, 1H, OH), 5.09 (t, 1H, J = 5.3Hz, C2'H), 4.90 (br, 1H, OH), 4.78 (br, 1H, OH), 4.27 (t, 1H, J = 5.3Hz, C3'H), 3.84 (m, 1H, C4'H), 3.71 (dd, 1H, J = 4.4Hz, J = -12.3Hz, C5'H), 3.55 (dd, 1H, J = 6.2Hz, J = -12.3Hz, C6'H).

4 K. Kohda, K. Baba, Y. Kawazoe, Tetrahedron 1990, 46, 1531-1540.
**Figure S1.** Variation of the absorbance at $\lambda = 398$ nm vs time after the addition of (a) 1 mM $H_2S$ or (b) Cys-SH to a 25 µM solution of 8-NO$_2$-Gua in phosphate buffer (100 mM, pH = 7.4, 200 µM DTPA) under aerobic (solid line) or anaerobic (dashed line) conditions at 37°C.

**Figure S2.** Evolution of the dioxygen concentration at 37°C after the addition of 10 mM $H_2S$ alone (solid line) or in the presence of 25 µM 8-NO$_2$-Gua (dashed line) in phosphate buffer (100 mM, pH = 7.4, 200 µM DTPA).
Figure S3. (a) HPLC traces recorded between 250 and 300 nm of (i) the crude reaction between 0.1 mM 8-NO₂-Gua and 1 mM H₂S in degassed Tris buffer (20 mM) containing 200 µM DTPA at pH 7.1 at 37°C for 3 h (●); (ii) the synthetic Gua-SH (●) and (iii) the starting Gua-NO₂ (●). (b) ESI+-HRMS spectrum of the main product observed by HPLC after the reaction between Gua-NO₂ and H₂S.
Figure S4. Cyclic voltammograms of 8-NO$_2$-Gua recorded at 20$^\circ$C on a glassy carbon electrode in deaerated 100 mM phosphate buffer. (a) and (b): effect of the pH on the reduction peak(s) at slow and high sweep rates. (c) and (d): effect of the sweep rate on the reversibility of peak I$_c$. 

\[ E \text{ vs Ag/AgCl (V)} \]

\[ E \text{ vs Ag/AgCl (V)} \]
Figure S5. ESI+-HRMS spectrum of the proposed intermediate disulfide.

Calculated m/z for $C_{15}H_{23}N_6O_7S_2\cdot H^+$: 463.1070 ($\Delta = 0.488$ ppm)
Calculated m/z for $C_{15}H_{23}N_6O_7S_2\cdot Na^+$: 485.0889 ($\Delta = 0.599$ ppm)

Figure S6. HPLC traces recorded between 250 and 300 nm and ESI+-HRMS spectra of the products of the reaction between cGMP-NO$_2$, H$_2$S and Pen-SH (100 µM, 1mM and 1 mM respectively, in 20 mM Tris buffer containing 200 µM DTPA at pH 7.1 at 37°C for 3 h).