Formation of glutathione sulfenate and sulfinate complexes by an organoiridium(III) anticancer complex

Zhe Liu[§], Peter J. Sadler*

Department of Chemistry, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, U.K.

[§]Current address: Department of Chemistry, University of Basel, Spitalstrasse 51, 4056 Basel, Switzerland

*e-mail: p.j.sadler@warwick.ac.uk

Support Information

Experimental

Figures S1 and S2

Materials. L-glutathione reduced (GSH), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), H_2O_2 (30%), quantofix peroxides test sticks, and catalase from bovine liver (aqueous suspension) were purchased from Sigma-Aldrich. ROS/superoxide detection kit was purchased from Enzo-Life sciences.

$[(\eta^5\text{-Cp}^{xbiph})\text{Ir(phpy)py}]^+$ (**Ir-py**) was synthesized as described previously.[1]

Methods and Instrumentation.

NMR Spectroscopy. ^1H NMR spectra were acquired in 5 mm NMR tubes at 298 K or 310 K on either a Bruker DPX 400 (^1H = 400.03 MHz) or AVA 600 (^1H = 600.13 MHz) spectrometers. ^1H NMR chemical shifts were internally referenced to CHD_2OD (3.33 ppm) for methanol-d_4. MeOD-d_4 was used to aid solubility. All data processing was carried out using MestReC or TOPSPIN version 2.0 (Bruker U.K. Ltd.).

Electrospray Ionization Mass Spectrometry (ESI-MS). Electrospray ionization mass spectra were obtained by using NMR samples for infusion into the mass spectrometer (Bruker Esquire 2000). The mass spectra were recorded with a scan range of *m/z* 400–1000 for positive ions.

pH Measurement. pH or pH* (pH meter reading without correction for effects of deuterium on glass electrode) values of NMR samples in H_2O or D_2O were measured at ca. 298 K directly in the NMR tube, before and after recording NMR spectra, using a Corning 240 pH meter equipped with a micro combination electrode calibrated with Aldrich buffer solutions of pH 4, 7 and 10.

Stability of Ir-SG in NaCl solution. NaCl was added to an NMR tube containing a ca. 0.8 mM solution of complex **Ir-SG** in 30% MeOD-d_4/70% D_2O to prepare a solution containing 104 mM NaCl. ^1H NMR spectra of the resulting solutions were recorded at 310 K and various time intervals for 17 h.

Interaction of Ir-py with GSH. GSH (3.5 mol equiv) was added to an NMR tube containing a 0.6 mM solution of complex **Ir-py** in 30% MeOD-d_4/70% PBS buffer

(H_2O) at ambient temperature, pH = 7.4. ^{1}H NMR spectra of the resulting solutions were recorded at 310 K and various time intervals for 12 h.

Interactions with NADH. NADH (2 mol equiv) was added to an NMR tube containing 95% **Ir-SG** and 5% **Ir-py**. ^{1}H NMR spectra of the resulting solutions at different conditions were recorded at 310 K.

Detection of H_2O_2. To a solution of **Ir-SG** (produced by reaction of 0.6 mM **Ir-py** with 3.5 mol equiv GSH in 30% MeOD-d_4/70% PBS H_2O buffer (v/v) at 310 K), 2 mol equiv NADH were added. After 48 h, H_2O_2 was detected by quantofix peroxide test sticks.

Oxidation of Ir-SG by H_2O_2. **Ir-SG** solution was prepared from the reaction of **1-py** (1 mM) with 3.5 mol equiv of GSH in 30% MeOD-d_4/70% PBS D_2O buffer (v/v) at 310 K, pH* = 7.4. H_2O_2 (10 mol equiv) was added to the above solution. ^{1}H NMR spectra of the resulting solutions were recorded at 310 K and various time intervals for 24 h.

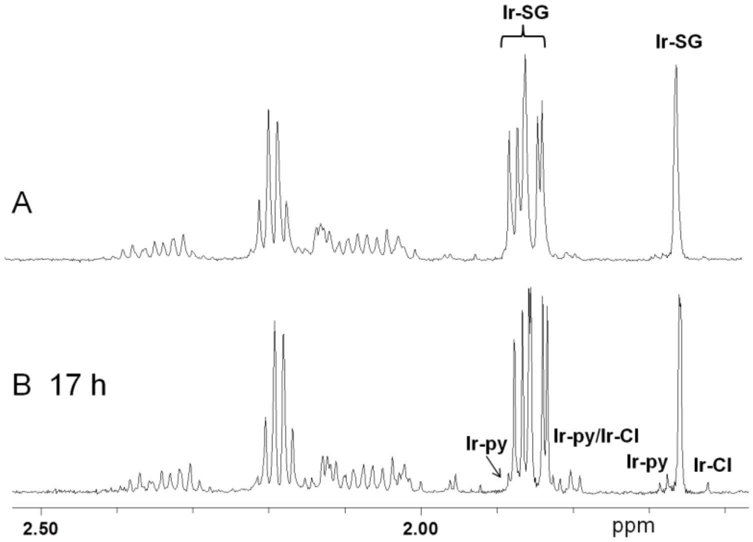

Figure S1. ^{1}H NMR spectra showing the stability of **Ir-SG** in the presence of 104 mM of NaCl. (A) Addition of NaCl (104 mM) to **Ir-SG** solution generated from the reaction between **Ir-py** (0.8 mM) and 3.5 mol equiv of GSH in 30% MeOD-d_4/70% phosphate D_2O buffer (v/v) at 310 K, pH* = 7.4. (B) 17 h later, 90% of the Ir was in the form of **Ir-SG** and 10% was present as **Ir-Cl** and **Ir-py**.

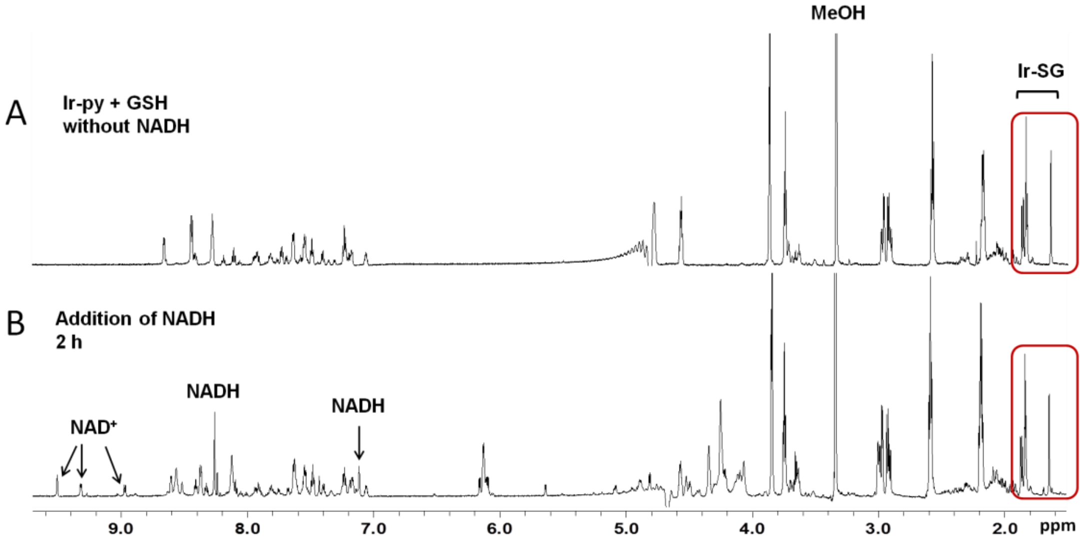

Figure S2. ^{1}H NMR spectra showing the reaction of NADH with **Ir-SG** in 30% MeOD-d_4/70% PBS H_2O buffer (v/v) at 310 K, deoxygenated under N_2. (A) Formation of **Ir-SG** produced by reaction of **Ir-py** (1.2 mM) with GSH (6 mM); (B) 2 h after addition of NADH (1.5 mol equiv) to the above equilibrium solution. The reaction produced NAD$^+$, however, no sulfenato complex **Ir-S(O)G** was generated. Peak assignments are shown. Little change occurs to the Cp^{xbiph} methyl peaks (red boxes).

Reference

(1) Liu, Z.; Romero-Canelón, I.; Qamar, B.; Hearn, J. M.; Habtemariam, A.; Barry, N. P. E.; Pizarro, A. M.; Clarkson, G. J.; Sadler, P. J. *Angew. Chem. Int. Ed.* **2014**, *53*, 3941-3946.