Oxidation induced by the antioxidant glutathione (GSH)

Holm Petzold and Peter J. Sadler*

Electronic supplementary information

Details of NMR-experiments

Schemes S1 and S2

Figure S1
Oxidation of [(η⁶-HMB)Ru(en)(S-iPr)]⁺ (1a) using GSH/air, maximum efficiency of oxygen transfer

Figure S2
Oxidation using GSSG/air

Figure S3
Competitive oxidation of [(η⁶-HMB)Ru(en)(S-iPr)]⁺ (1a) and GSH with H₂O₂

Figure S4
Oxidation of [(η⁶-p-cymene)Ru(en)(S-Ph)]⁺ (1b) with GSH/air
Oxidation reactions

GSH in aqueous solution at pH ca. 7 undergoes very slow oxidation to GSSG. On their own in aqueous solution, complexes 1a and 1b are stable, even on bubbling with air or oxygen for several hours. In contrast, these complexes are readily oxidized to sulfenates within a few hours (1 - 4 h) when GSH is present in solution at the same time; the GSH is oxidized to GSSG and the reactions are accelerated by bubbling with air. The reactions can be readily followed by NMR spectroscopy, but not monitored whilst bubbling with air. Longer reaction times lead to a displacement of the en ligand, probably by GSH or GSSG, and to subsequent decomposition of 1a and 1b.

Details of NMR-Experiments

1H NMR spectra were recorded on a Bruker ava600 spectrometer equipped with a triple resonance TXI (1H, 13C, 15N) z-gradient cryo-probe or room temperature TXI (1H, 13C, 15N) triple-axis (x, y, z) gradient probehead. Spectra were recorded at 298 K, unless otherwise stated. Suppression of the water signal for 90% H2O/10% D2O samples was achieved by presaturation or a double-pulsed field-gradient spin-echo routine (DPFGSE). 1H NMR spectra were referenced to dioxane as internal standard (3.71 ppm). 15N chemical shifts were referenced to NH4OH as external standard.

Scheme S1: The characteristic β-CH2 NMR resonances of the thiol/disulfide used to assign the redox-state of GSH/GSSG. For reported assignments see references 26-28.
Scheme S2: Protons (green and orange) whose NMR resonances were used to monitor the thiolato complexes 1a, 1b and sulfenato complexes 2a, 2b during the oxidation reactions.10
Oxidation of \([\eta^6\text{-HMB})\text{Ru(en)(S-} \text{-iPr)}]^{+}\) (1a) using GSH/air, maximum efficiency of oxygen transfer.

**Figure S1**: Stacked plot of \(^1\text{H-NMR spectra for a solution of complex 1a (0.5 mM) and GSH (2.5 mM). a) After mixing, and b) after bubbling air for 4 h, and c) after additional bubbling with air overnight.} After the complete oxidation of GSH, most of the complex is oxidized to the sulfenato complex \([\eta^6\text{-HMB})\text{Ru(en)(S(O)-} \text{-iPr)}]^{2+}\) 2a together with a small amount of decomposition of products from the starting material. Note that for every transferred oxygen atom, two molecules of GSH are oxidized to one molecule of GSSG and one water molecule is formed; therefore oxygen transfer is remarkably efficient, ca 35% of the theoretical value. For assignments see Schemes S1 and S2 (colour code: 1a, 2a, GSH, GSSG).

**Oxidation using GSSG/air**

**Figure S2**: When air was bubbled through a solution of complex 1a (0.5mM) and GSSG (1.25 mM), no oxidation to the sulfenato complex was observed; instead displacement of the en ligand occurred with subsequent formation of various unidentified complexes. For assignments see Schemes S1 and S2 (colour code: 1a, 2a, GSSG, unidentified complexes).
Competitive oxidation of $[(\eta^6\text{-HMB}) \text{R(en)} (\text{S-}i\text{Pr})]^+$ (1a) and GSH with H$_2$O$_2$

**Figure S3:** When H$_2$O$_2$ (1 mol equiv with respect to GSH) is added to a solution containing GSH (1 mM) and complex 1a (0.5 mM), complex 1a reacts faster than GSH. For assignments see Schemes S1 and S2 (1a, 2a, GSH, GSSG).

**Oxidation of $[(\eta^6\text{-p-cymene}) \text{Ru(en)}(\text{S-Ph})]^+$ (1b) with GSH/air**

**Figure S4:** $^1$H NMR spectra recorded during reactions of 0.5 mM complex 1b with 2.5 mM GSH with air-bubbling for 5 h. Complete oxidation of GSH to GSSG has occurred; oxidation of complex 1b is incomplete, but still detectable. The phenyl substituent on the thiolate in 1b is a weaker electron donor compared to the isopropyl group in 1a and together with the presence of a weaker arene donor ($p$-cymene compared to hexamethylbenzene) accounts for the slowing down of the reaction. For assignments see Schemes S1 and S2 (1b, 2b).

**References**