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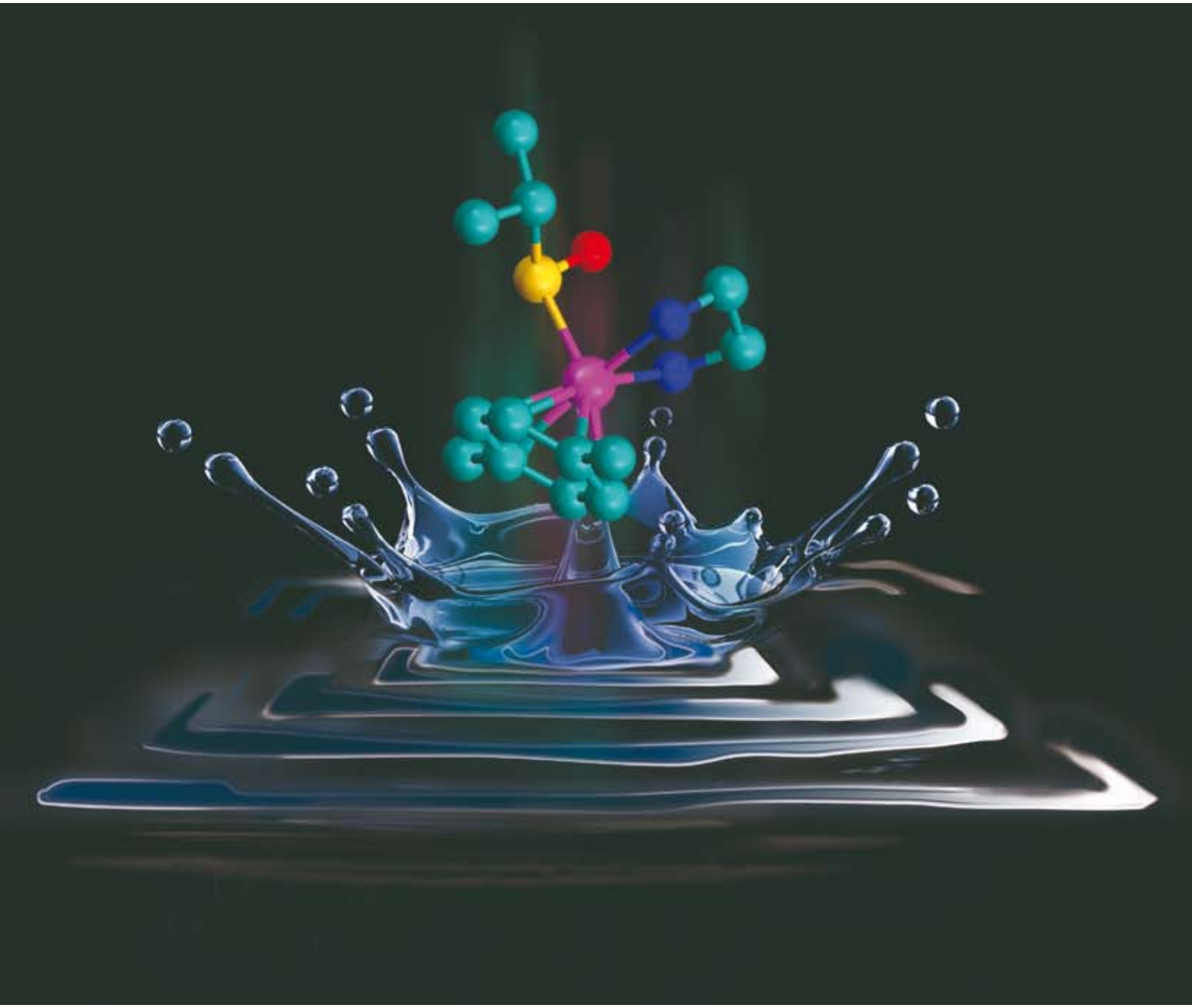

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# Oxidation induced by the antioxidant glutathione (GSH)†

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**Remarkably efficient oxygen atom transfer from an intermediate of glutathione (GSH) autoxidation to an organometallic ruthenium arene thiolato complex is observed under physiologically-relevant conditions.**

The tripeptide glutathione (γ-L-Glu–L-Cys–Gly, **GSH**, Fig. 1) is frequently the most abundant cytosolic thiol compound, with concentrations in the range of 0.1–10 mM.[1–3] Glutathione serves as substrate for GSH-peroxidase.[4] Inside cells, a constant ratio of **GSH**/**GSSG** (*ca.* 10) is maintained by reduction of **GSSG** to **GSH** by glutathione disulfide reductase.[2,5] Notably the oxidation of **GSH** by air *in vitro* exhibits a maximum rate at pH = 9.[6]

Glutathione plays a major role in intracellular drug metabolism, especially in detoxification and drug resistance. GSH conjugates with drugs can be pumped out of cells by specific membrane transport systems, and sulfur-bound thiolate adducts with platinum anticancer drugs are formed irreversibly and are largely unreactive (*e.g.* towards DNA binding).[7]

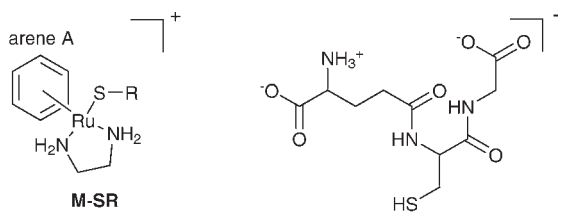
Our interest in organometallic $Ru^{II}$ arene anticancer complexes[8] of the type $[(\eta^6\text{-arene})Ru(diamine)Cl]^+$ has led us to investigate the reactivity of thiolato ($RS^-$) adducts $[(\eta^6\text{-arene})Ru(en)(SR)]^+$ (en = ethylenediamine).[9] Our initial studies have suggested that they may not be inert but can undergo activation by ligand-based redox reactions.

Here we report studies of the oxidation of thiolato complexes of the type **M–SR** (M = ($\eta^6$-arene)Ru(en), arene = hexamethylbenzene (HMB) **1a** or *p*-cymene **1b**, Fig. 1) by the couple oxygen–GSH under physiologically-relevant conditions.‡ Recently we have shown that the sulfenato complex **2a** (**M–S(O)R**) can hydrolyze to form reactive aqua complexes **M–OH$_2$**.[10] The GSH adduct **M–SG**, which may be formed *in vivo*, undergoes facile oxidation to the sulfenato complex **M–S(O)G** in air.[11] In contrast, complex **1** containing isopropylthiolate or thiophenylate (**M–SR**, R = Ph, *i*Pr) is not sensitive to air, but is oxidized by hydrogen peroxide stepwise to the respective sulfenato complex **2** (**M–S(O)R**) and the sulfinato complex **M–S(O)$_2$R**.[10]

To our great surprise, during monitoring of the competitive reaction of the thiolato complex **1a** with guanosine 3′,5′-cyclic monophosphate (GMP) and in the presence of **GSH**, we observed formation of the sulfenato complex **2a M–S(O)R**, although the oxidation was incomplete and most of the complex formed products which had lost the en-ligand.[12] Incomplete oxidation of **1** (**M–SR**, 1–2 mM) is not surprising because oxygen is sparingly soluble in water under the conditions used (6.9 mg $l^{-1}$under an atmosphere of 1 bar air, *ca.* 0.21 mM).[13] When air was bubbled through a reaction mixture (10 mM **GSH**, 1 mM **1a**, 2.7 mM KCl and 13.7 mM NaCl, 10 mM phosphate buffer, pH = 7), the oxidation was much faster and, after several hours at ambient temperature, complete oxidation of **1a** to **2a** was observed (Fig. 2, Scheme 1). At the same time **GSH** was almost completely oxidized to **GSSG**. When the reaction was carried out with **GSSG** instead of **GSH** no oxidation of **M–SR** was observed (Fig. S2†).

The transfer of oxygen is surprisingly efficient, with a conversion of up to *ca.* 0.35 mol complex per mol $O_2$. As few as 5 molar equivalents of **GSH** were enough to induce oxidation of most of complex **1a** to the sulfenato complex **2a** (Fig. S1†). Evidently, during the oxidation of **GSH** by oxygen, a kinetically-more-reactive species than $O_2$ is formed. Such a species could be the peroxo sulfenic acid **GSOOH**, which could give rise to formation of hydrogen peroxide *via* condensation with **GSH** to give **GSSG** (Scheme 2).[14] Formation of $H_2O_2$ or, in general, reactive oxygen species (ROS) during the autoxidation of glutathione has been reported.[15] The formation of peroxo species is known to occur during the oxidation of thiolato complexes and sulfides, as well as disulfides, with singlet oxygen.[16–21] Transfer of oxygen atoms from an initial peroxo species formed by reaction of a thioether with singlet oxygen to another thioether has been observed in well selected thioether couples.[21]

If this pathway prevails, then addition of hydrogen peroxide should lead to the preferential oxidation of the thiolato complex **1a** instead of **GSH**. Indeed when $H_2O_2$ was added to a solution of **GSH** and **1a** at pH = 7.5, preferential oxidation of **1a** was observed (Fig. S3†).




**1a** A = $\eta^6$-HMB, R = *i*Pr;
**1b** A = $\eta^6$-*p*-cymene, R = Ph;

**Fig. 1** Structures of complexes used in this study and of **GSH** in its ionic state at physiological pH (close to pH 7).[6]

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† Electronic supplementary information (ESI) available: Schemes S1 and S2, and details of NMR experiments, Fig. S1–S4. See DOI: 10.1039/b805358h

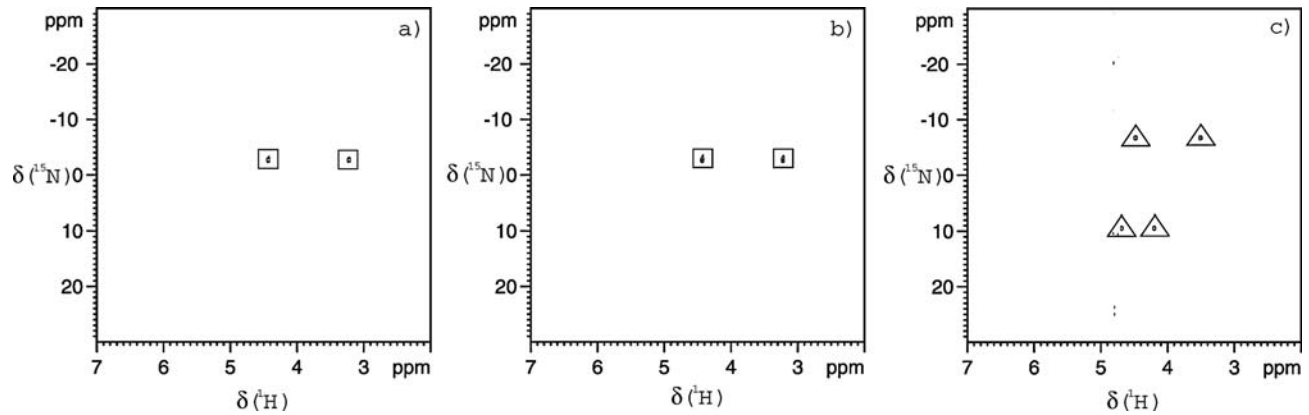
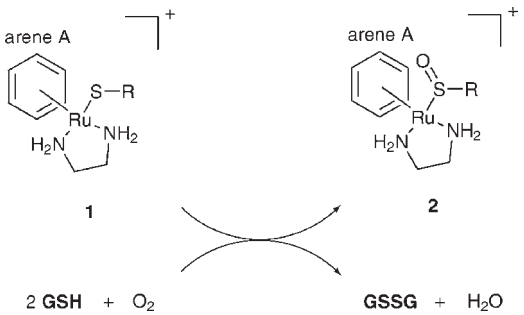
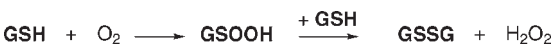



**Fig. 2** Complex $[(\eta^6\text{-HMB})Ru(en)(S\text{–}i\text{Pr})]^+$ (**1a**) is stable in air, but is oxidized by **GSH**–air. $^1H$, $^{15}N$ HSQC NMR spectra. (a) Control: 1 mM complex **1a** (□), and (b) the same solution after bubbling air through for 4 h; no oxidation is observed. Even prolonged air-bubbling did not lead to oxidation of **1a** in the absence of **GSH**. (c) Solution of 1 mM complex **1a** and 10 mM **GSH** after bubbling air through for 4 h; the signals correspond to the sulfenato complex $[(\eta^6\text{-HMB})Ru(en)S(O)\text{–}i\text{Pr})]^+$ (△) (**2a**). For oxidation of **GSH** on its own see ref. 6.




**Scheme 1** Oxidation of complex **1a** is observed only in the presence of **GSH**–$O_2$.




**Scheme 2** Possible mechanism[14] for the formation of hydrogen peroxide from peroxo glutathione sulfenic acid by hydrolysis or condensation with **GSH**.

The efficiency of oxygen transfer depends on the nature of the thiolato ligand in **M–SR**. Complex **1b**, which bears an electron-withdrawing phenyl group instead of the electron-donating isopropyl group in **1a**, is oxidized to **2b** to a smaller extent (Fig. S4†).

In summary, our data show that **GSH** can act as a source of reactive oxygen species, able to induce oxidation of molecules which are themselves stable in air. The efficiency of oxygen transfer of up to 0.35 mol per mol $O_2$–2**GSH** is remarkable, especially under near physiological conditions. It seems unlikely that this reaction is restricted to thiolato complexes of the type **M–SR** (**1**) used in this work. The impact of this ROS in the cell may be restricted by the presence of catalases which decompose peroxides, but ROS are always present in cells and their levels are significantly increased in cancer cells. If thiolato complexes react much faster with such species than catalases can decompose ROS, then activation by oxidation inside cells is likely, as has been postulated for other drugs such as diallyltrisulfide.[22,23]

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## Notes and references

‡ Starting materials $[(\eta^6\text{-HMB})Ru(en)(S\text{–}i\text{Pr})]PF_6$ (**1a**) and $[(\eta^6\text{-}p\text{-cymene})Ru(en)(S\text{–Ph})]PF_6$ (**1b**) were prepared according to the literature procedure.[10] All aqueous samples contained 2.7 mM KCl and 13.7 mM NaCl as well as 10 mM phosphate buffer to stabilize the pH at 7.4. For bubbling air, a syringe needle was fitted with a balloon and with some filters as a pressure reducer; the air flow was *ca.* 1 l $h^{-1}$. For all samples with less than 5 mM **GSH**, the pH was almost constant during bubbling, and was checked using an indicator. Mixtures of **GSH** were prepared as 100 mM or 25 mM stock solutions and were adjusted to pH 6–8 by adding NaOH and used within a few hours to avoid initial oxidation. The NMR sample volume was 0.6 ml, and HPLC grade water was used. **GSH** and **GSSG** were purchased from Sigma-Aldrich.

1 A. Corazza, I. Harvey and P. J. Sadler, *Eur. J. Biochem.*, 1996, **236**, 697.
2 A. Meister, *J. Biol. Chem.*, 1988, **263**, 17205.
3 A. Meister and M. E. Anderson, *Annu. Rev. Biochem.*, 1983, **52**, 711.
4 B. Halliwell and J. M. C. Gutteridge, *Free radicals in biology and medicine*. Oxford University Press, Oxford, 1999.
5 A. Meister, A brief history of glutathione and a survey of its metabolism and functions, , in *Coenzymes and Cofactors*, ed. D. Dolphin, R. Poulson and O. Avramovic, Wiley, New York, 1988, pp. 1–48.
6 A. Krezel, W. Szczepanik, M. Sokołowska, M. Jezowska-Bojczuk and W. Bal, *Chem. Res. Toxicol.*, 2003, **16**, 855.
7 J. Reedijk, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3611.
8 Y.-K. Yan, M. Melchart, A. Habtemariam and P. J. Sadler, *Chem. Commun.*, 2005, 4764.
9 F. Wang, A. Habtemariam, E. P. L. van der Geer, R. Fernández, M. Melchart, R. J. Deeth, R. Aird, S. Guichard, F. P. A. Fabbiani, P. Lozano-Casal, I. D. H. Oswald, D. I. Jodrell, S. Parsons and P. J. Sadler, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 18269.
10 H. Petzold, J. Xu and P. J. Sadler, *Angew. Chem., Int. Ed.*, 2008, **47**, 3008.
11 F. Wang, J. Xu, A. Habtemariam, J. Bella and P. J. Sadler, *J. Am. Chem. Soc.*, 2005, **127**, 17734.
12 F. Wang, H. Chen, J. A. Parkinson, P. d. S. Murdoch and P. J. Sadler, *Inorg. Chem.*, 2002, **41**, 4509.
13 P. A. Vesilind, *Introduction to environmental engineering*, PWS Publishing Company, Boston, 1996.
14 Other possible reaction pathways could involve formation of superoxide radicals ($O_2^-$) *via* thiyl radicals ($RS^\bullet$); for a general review see ref. 22 and literature cited therein.

 

15 T. V. Votyakovaa and I. J. Reynolds, *Arch. Biochem. Biophys*., 2004, **431**, 138.
16 C. Galvez, D. G. Ho, A. Azod and M. Selke, *J. Am. Chem. Soc*., 2001, **123**, 3381.
17 C. A. Grappenhaus and M. Y. Darensbourg, *Acc. Chem. Res*., 1998, **31**, 451.
18 W. Weigand, G. Bosl, B. von Dielingen and K. Gollnick, *Z. Naturforsch., B: Chem. Sci*., 1994, **49**, 513.
19 S. M. Bonesi, M. Fagnoni, S. Monti and A. Albini, *Tetrahedron*, 2006, **62**, 10716.
20 E. L. Clennan and C. Liao, *Tetrahedron*, 2006, **62**, 10724.
21 F. Jensen, A. Greer and E. L. Clennan, *J. Am. Chem. Soc*., 1998, **120**, 4439.
22 U. Muenchberg, A. Anwar, S. Mecklenburg and C. Jacob, *Org. Biomol. Chem*., 2007, **5**, 1505.
23 C. Jacob, *Nat. Prod. Rep*., 2006, **23**, 851.