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Palladium(II) diamine complex induces reduction of glutathione disulfide†

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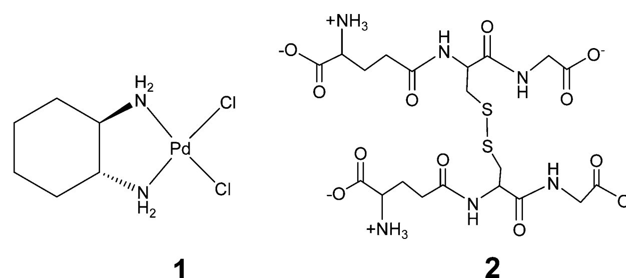
Reaction of [Pd(1*R*,2*R*-diaminocyclohexane)Cl_2] with the oxidised form of the tripeptide glutathione (γ-L-glutamyl-L-cysteinyl-glycine) in aqueous solution leads to reductive cleavage of the disulfide bond.

Diam(m)ino Pt^{II} complexes are now well established in the clinic as anticancer drugs.[1] There has been much interest in the possibility of designing active Pd^{II} analogues,[2] on account of the tendency of Pd^{II} to form similar square-planar complexes. However, this approach has met with very limited success on account of the much higher kinetic lability of Pd^{II}.[3] Here we report a novel redox reaction of [Pd(1*R*,2*R*-dach)Cl_2] **1**, where dach = 1,2-diaminocyclohexane, with the biologically important molecule glutathione disulfide. The Pt^{II} analogue [Pt(1*R*,2*R*-dach)Cl_2] is a potent anticancer agent, but **1** exhibits little cytotoxicity to cancer cells.[4] The tripeptide glutathione (GSH) is present in cells at concentrations as high as 10 mM and a small percentage is present as the oxidised form GSSG (**2**).[5] Unexpectedly we have observed cleavage of the disulfide bond of GSSG by **1**. There appear to be no other reports of the reduction of disulfides by Pd^{II} complexes.

First, we studied the reaction of **1** (0.1 mM) with GSSG (0.2 mM) at pH 7, 310 K, by UV–vis spectroscopy. Two relatively intense bands (ε > 2000–4000 M^{-1} cm^{-1}) appeared at 267 and 348 nm and continued to increase in intensity over a period of more than 48 h, Fig. 1, with a slower second phase following a first phase of *ca.* 10 h. Curiously, in more highly acidic solutions (pH 1.5) little reaction between **1** and GSSG was observed by UV–vis spectroscopy, even after 48 h.

The reaction of **1** (0.1 mM) with GSH (0.2 mM) at pH 7, 310 K, also gave rise to a band at 267 nm with similar intensity to that observed with GSSG (ε *ca.* 9000 M^{-1} cm^{-1}; possibly a thiolate S-to-Pd^{II} charge-transfer band[6]) together with additional bands at 277, 320 and 391 nm (Fig. S2†), and was complete in *ca.* 8 h.



In order to identify the products from the reaction of **1** with GSSG, reverse-phase HPLC studies were carried out using a C_{18} column‡. One major product was separable after 25 h of reaction, Fig. 2a (peak C). The mass spectrum of the HPLC fractions corresponding to peak C showed a cluster of peaks which are well

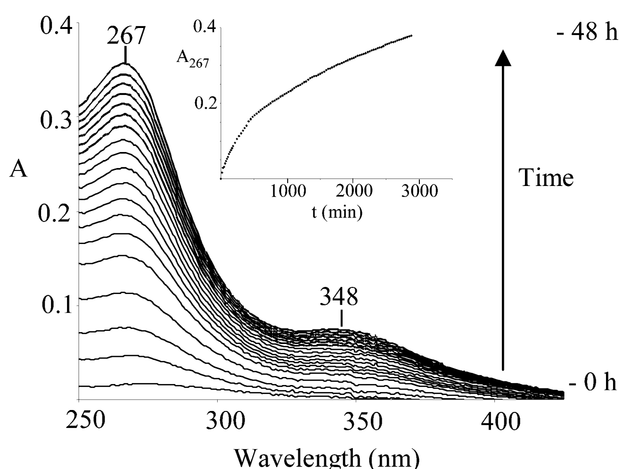


Fig. 1 UV–vis spectra recorded during the reaction of [Pd(1*R*,2*R*-dach)Cl_2] **1** (0.1 mM) with GSSG (0.2 mM) at pH 7, 310 K. The inset shows the variation in A_{267} with time.

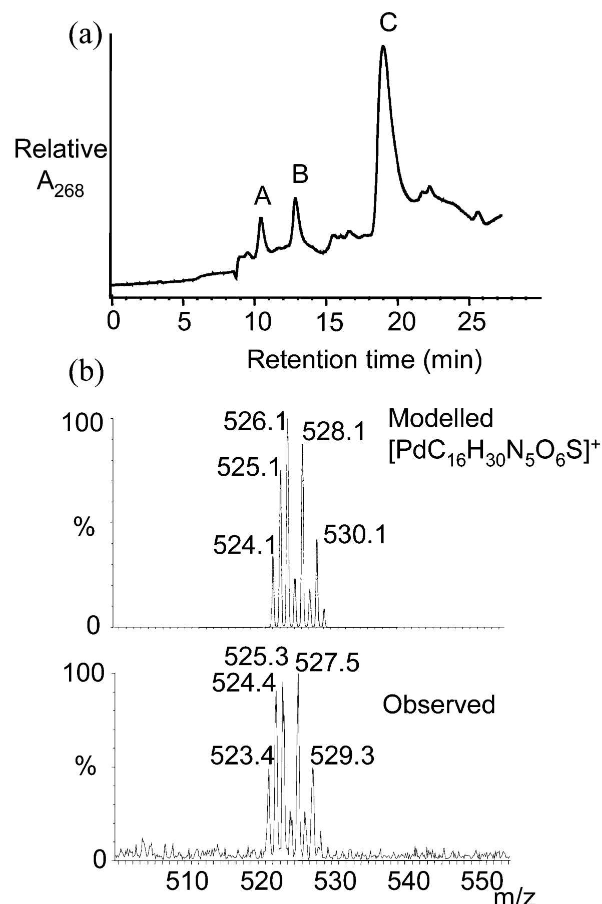


Fig. 2 (a) HPLC separation of the species from reaction of [Pd(1*R*,2*R*-dach)Cl_2] **1** (0.1 mM) with GSSG (0.2 mM) at pH 7, 310 K, for 25 h on a C_{18} column eluted with a gradient of CH_3CN–H_2O containing 0.1% TFA as ion-pairing agent. (b) Observed mass spectrum of peak C of the HPLC chromatogram, and calculated isotopic splitting pattern for $[PdC_{16}H_{30}N_5O_6S]^+$.

† Electronic supplementary information (ESI) available: experimental details, UV–vis spectra for GSH reaction, 2D TOCSY spectrum of **3**. See http://www.rsc.org/suppdata/cc/b4/b408151j/

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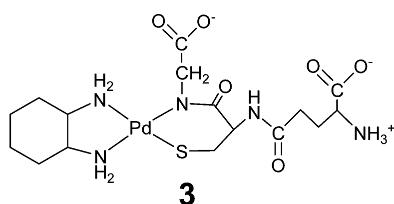
modelled as $[PdC_{16}H_{30}N_5O_6S]^+$ (*m/z* 525.8; [**3** + $2H^+$]), as shown in Fig. 2b.

Further information about the identity of complex **3** was obtained by 800 MHz ^{1}H NMR spectroscopy. The 2D TOCSY spectrum showed the presence of one independent spin system for both dach and glutathione protons. The absence of a peak for glycinyl NH and the large low-field shift of the cysteinyl α(CH) (δ 5.38), as well as the UV–vis and mass spectrometry data, are consistent with a structure for **3** in which the dach ligand remains bound to Pd^{II} and the Cl/H_2O ligands have been substituted by GS (arising from reduction of GSSG), chelated *via* the thiolate S and deprotonated glycinyl amide N. The chemical shifts of **3** are compared to those of GSSG in Table S1.†



Reductive cleavage of a disulfide bond by a Pd^{II} complex appears to be a very unusual reaction. Our literature searches revealed no other examples. Pd^{II} diamine complexes are known to cleave peptide bonds involving methionine and histidine residues efficiently, especially under acidic conditions.[7] Reactions of diamino Pd^{II} complexes such as $[Pd(1,2\text{-diaminoethane})(H_2O)_2]^{2+}$ with GSH have been reported to give rise to polymeric products.[8] Thus GSH adducts formed *via* reductive cleavage of GSSG appear to be novel species.

In view of the strongly oxidising nature of Pd^{III} and Pd^{IV}, it seems likely that the electrons required for reduction of GSSG by **1** are not supplied directly by Pd^{II}, but instead by coordinated aqua/hydroxo ligands (Pd-OH/OH_2). Complex **1** undergoes rapid hydrolysis in aqueous solution and the pK_a values of aqua ligands on $\{Pd(diamine)\}^{2+}$ are low (*e.g.* 6 and 7 for $[Pd(en)(H_2O)_2]^{2+}$).[9] Moreover it has been reported that GSSG undergoes partial reduction in strong alkali (0.2 M KOH).[10] Initial coordination of GSSG to Pd^{II} *via* the disulfide bond would provide a pathway for direct electron transfer from coordinated OH/OH_2. Disulfide coordination to Pd^{II} is known, for example in complexes of the cyclic peptide oxytocin.[11]

We found that solutions of **1** and GSSG under the conditions of the UV–vis experiments contained *ca.* 29 μM H_2O_2 after 25 h.§ The formation of *ca.* 0.5 mol of H_2O_2 per mol of product **3** is consistent with Pd–(H_2O/OH) acting as a one-electron reducing agent. It is possible therefore that a 5-coordinate intermediate with two bound H_2O/OH ligands such as $\{Pd(1R,2R\text{-dach})(GSSG\text{-}S)(H_2O/OH)_2\}$ is involved in the reaction. We considered the possibility that other oxidised glutathione species such as sulfinic and sulfenic acids may also be products of the reaction, but attempts to assign peaks to them in the mass spectrum of the reaction mixture were inconclusive. Oxidation of metal-coordinated aqua ligands is of much interest in relation to the production of dioxygen by the photosynthetic reaction centre[12] and perhaps may be significant at other biological metal centres. Cu^{II}-promoted reduction of GSSG has been reported.[13] The Pt^{II} analogue of **1** also reductively cleaves GSSG but the major products are dinuclear thiolate S-bridged 2 : 1 and 2 : 2 Pt^{II}:GS species.[14] Water is also thought to provide the electrons involved in the reduction of disulfides by Pt^{II} amines.[15] The ability of coordinated aqua ligands to act as reductants is a property which requires wider consideration in the design of biologically-active metal complexes.

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Notes and references

‡ *Experimental details*: high performance liquid chromatography (HPLC) was carried out on a Hewlett Packard 1100 system with UV detection (268 nm) using a RP C_{18} column (250 × 4.6 mm, 100 Å, 5 μM, Hichrom), eluting with a 0–50% acetonitrile gradient in water (+ 0.1% trifluoroacetic acid as an ion-pairing agent).

Electronic absorption spectra were recorded over time on a CARY 300 Scan UV–vis spectrometer fitted with a temperature controller over a range of 800–200 nm at 310 K.

1D and 2D ^{1}H NMR spectra were recorded at 298 K in 5 mm NMR tubes on Bruker DMX 500 MHz or Bruker AVA 800 MHz spectrometers, using dioxane (δ(^{1}H) 3.76 ppm) as an internal standard.

All pH measurements were recorded at 298 K directly in the NMR tube, prior and following NMR measurements, using a Corning 145 pH meter equipped with a chloride-free micro combination electrode calibrated with Aldrich standard buffers (pH 4, 7 and 10). The pH was adjusted with dilute solutions of $HClO_4$ and NaOH.

Positive ion electrospray mass spectrometry (ESI-MS) was performed on a Platform-II mass spectrometer (Micromass, Manchester, UK), at a cone voltage of 30 V.

Molecular modelling was carried out using SYBYL (version 6.9, Tripos, Inc.). Models were energy-minimised using the Tripos force field in the drop-down menu. Models were considered energy-minimised when a constant energy was achieved.

Typical reactions of palladium complex **1** were carried out as follows. A solution containing freshly-dissolved $[Pd(1R,2R\text{-dach})Cl_2]$ in water (100 μM, 2 mL, 0.2 μmol) was added to an aqueous solution of GSH or GSSG (20 mM, 10 μL, 0.2 μmol, pH 7) and the reaction mixture was incubated at 310 K for 24 or 48 h. The reaction was monitored using HPLC and UV–vis techniques.

§ H_2O_2 was detected using "Quantofix" Peroxide Test Sticks (Machery-Nagel). This value was determined by the average from triplicate experiments. No H_2O_2 was detected in reactions of **1** with GSH.

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