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Tryptophan Switch for a Photoactivated Platinum Anticancer Complex

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Supporting Information

ABSTRACT: The octahedral Pt^{IV} complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**) is potently cytotoxic to cancer cells when irradiated with visible (blue) light. We show that the acute photocytotoxicity can be switched off by low doses (500 μM) of the amino acid L-tryptophan. EPR and NMR spectroscopic experiments using spin traps show that L-Trp quenches the formation of azidyl radicals, probably by acting as an electron donor. L-Trp is well-known as a mediator of electron transfer between distant electron acceptor/donor centers in proteins, and such properties may make the free amino acid clinically useful for controlling the activity of photochemotherapeutic azido Pt^{IV} drugs. Since previous work has demonstrated the ability of photoactivated **1** to platinate DNA, this suggests that the high potency of such photoactive platinum complexes is related to their dual attack on cancer cells by radicals and Pt^{II} photoproducts.

We report that the essential amino acid L-tryptophan (L-Trp) can quench the formation of azidyl radicals from the potent photochemotherapeutic anticancer complex *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**, py = pyridine; Figure 1)

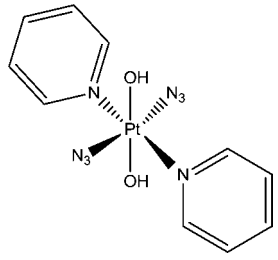

Figure 1. *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(py)_2]$ (**1**).

and suppress inhibition of cell growth at low, physiologically relevant doses. These new data not only establish an acute radical-based mechanism of action for photoactivatable Pt^{IV} anticancer complexes but also suggest a means of switching off this activity to avoid unwanted side effects.

The platinum-based anticancer drugs cisplatin, carboplatin, and oxaliplatin are widely used in the clinic, but they are untargeted, and their use is often accompanied by side effects.[1,2] Photoactivation offers a possible mechanism for specifically targeting the anticancer activity of metal compounds.[3a−c] Successful strategies involving the photoactivation of stable prodrugs can give rise to the release of bioactive ligands or cleavage and binding to DNA.[4a−i] Suitable complexes include stable Pt^{IV} complexes that are inert and unreactive under thermal conditions in the dark but can be activated in cancer cells by light.[5] Promising complexes include the diazido Pt^{IV} complexes $[Pt(N_3)_2(OH)_2(am_1)(am_2)]$, where am_1 and am_2 are amine ligands such as NH_3 or pyridine.[6a−c] A particularly potent compound that can be activated by UVA, blue, and green light is the all-trans complex **1**, which is highly active against a range of cancer cell lines, including cisplatin-resistant A2780 human ovarian carcinoma cells.[7] Such diazido complexes can platinate DNA and produce lesions that are distinctly different from those generated by cisplatin.[8] The pyridine ligands appear to remain strongly bound even after photoreduction to Pt^{II}, and the major DNA adducts of photoactivated **1** efficiently stall RNA polymerase II.[9] The aim of the present work was to investigate the fate of the azido ligands, in particular the possibility of photoinduced release of azidyl radicals.

First, an aqueous solution of **1** (5 mM) was irradiated with blue light (463 nm, 64 mW cm^{-2}) in the presence of a 2-fold molar excess of the spin trap 5,5-dimethylpyrroline *N*-oxide (DMPO). This generated a quartet of triplets in the X-band electron paramagnetic resonance (EPR) spectrum (Figure 2A)

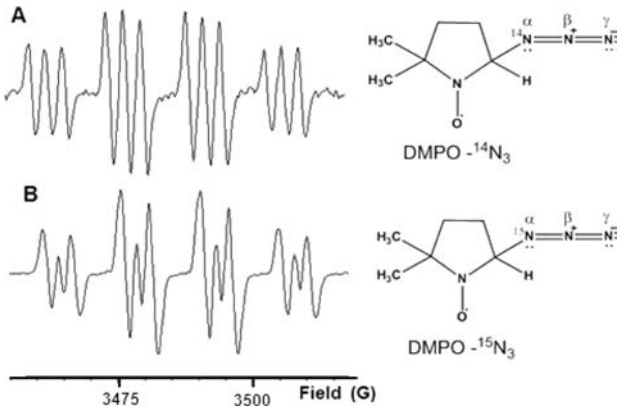

Figure 2. X-band EPR spectra of the products of photoactivation with blue light (463 nm, 64 mW cm^{-2}) of 5 mM aqueous solutions of (A) complex **1**, and (B) [^{15}N]**1** (50% ^{15}N at N_α) in the presence of 2 molar equiv of the spin trap DMPO.

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assignable to the azidyl adduct of DMPO (a^{N}_{NO} = 14.5 G, a^{H}_{β} = 14.9 G, a^{N}_{α} = 3.16 G).[10] No radicals were trapped in the dark control sample [Figure S1 in the Supporting Information (SI)]. To confirm that the radicals arose from Pt^{IV}-bound azide, the experiment was repeated with [^{15}N]**1** prepared from azide labeled with ^{15}N at one of the terminal nitrogen atoms ([^{15}N═N═N]$^{-}$). This generated the spectrum in Figure 2B, which contains a quartet of doublets[11] overlapped by peaks from residual [^{14}N]**1** (50% ^{14}N at the N_{α} position) (Table S1 in the SI), as confirmed by simulation of the spectra of trapped [^{14}N]- and [^{15}N]azidyl radicals (Figure S2). There appear to be no previous reports of azidyl radical trapping from a Pt^{IV} complex, although formation of azidyl radicals accompanied by one-electron oxidation of the metal is a known pathway for the photodecomposition of some other metal azido complexes, including mixed-ligand complexes of Pt, Pd, Fe, and Au.[12a−e]

The concentration of the trapped radical species DMPO-$^{14}N_3$ after irradiation of aqueous **1** (5 mM) and DMPO (10 mM) at ambient temperature increased during 14 min of continuous irradiation to a maximum of 474 μM (using Tempol as a standard), after which time the concentration gradually decayed to 116 μM over the next 70 min under continued irradiation. When the concentration of **1** was lowered to 150 μM, the maximum yield of trapped azidyl radicals (after 14 min of irradiation) was 90 μM (i.e., ca. 51% higher per mol of complex **1** compared to the yield above with the higher concentration of **1**), perhaps consistent with azidyl radical dimerization at high concentrations to produce N_2 as a competing decomposition pathway. N_2 can be detected as a photodecomposition product of **1** (20 mM in D_2O) by ^{14}N NMR spectroscopy.[13]

Next, the influence of the solvent was investigated. Radicals were also trapped after irradiation of 5 mM **1** in phosphate-buffered saline (PBS) and in the culture medium RPMI-1640. Interestingly, the yield of azidyl radicals in PBS was 541 μM, which is higher than in water alone. However, when the irradiation wavelength was increased to 517 nm (green light), radicals were trapped only in the RPMI culture medium (Table S2). The reason why activation at the longer wavelength was successful only in the culture medium is not clear, although perhaps it could be related to the behavior of very weak bands at longer wavelength found in density functional theory calculations.[7] RMPI medium contains a variety of amino acids, vitamins, inorganic salts, and other growth-promoting substances,[14] which may be responsible for the enhanced release of the azidyl radicals at longer wavelengths. Longer wavelengths are of interest because of their deeper penetration into tissues, and both blue and green light are effective in activating related pyridyl/ammine Pt^{IV} complexes in cancer cells.[6a,7]

We then investigated the effect of biologically relevant electron donors on the formation of azidyl radicals from the photoactivation of **1**. The strongest electron donor in DNA is the base guanine, but surprisingly, the presence of 2 molar equiv of 5′-guanosine monophosphate (GMP) had little effect on the production of azidyl radicals from **1** induced by blue light (see the SI). No precipitate formed in the presence of GMP, in contrast to its absence, consistent with electron transfer from the azido ligands to Pt^{IV} to give azidyl radicals together with formation of soluble Pt^{II}−GMP photoproducts, as we have observed previously by NMR spectroscopy.[15]

In proteins, the side chains of the amino acids tyrosine and tryptophan are known to be efficient mediators of electron transfer between distant redox centers.[16] Both Trp$^{\bullet}$ and TyrO$^{\bullet}$ radicals are strong oxidants. At pH 7, Trp$^{\bullet}$ (E° = 1.015 V vs NHE) is a slightly stronger oxidant than TyrO$^{\bullet}$ (E° = 0.93 V vs NHE) but weaker than the azidyl radical (E° = 1.33 V vs NHE). These reduction potentials, together with the pK_a values[17,18] of 4.74 for HN_3 and 4.3 for TrpH$^{\bullet+}$, suggest that it is favorable for L-Tyr and L-Trp to transfer an electron to the azidyl radical.[19] The redox potentials of both L-Trp and L-Tyr show a marked pH dependence.[20]

Surprisingly, the presence of L-Tyr (up to 1 mM) had little effect on the trapping of azidyl radicals by DMPO (see the SI). However, L-Trp was effective in suppressing radical trapping, even at low physiologically relevant concentrations. The presence of 1 mM L-Trp completely suppressed the EPR trapping of azidyl radicals in a 4 mM solution of **1** in PBS irradiated with blue light, and 0.125 molar equiv (500 μM) suppressed radical formation by ca. 70% (Figure 3). Next, we

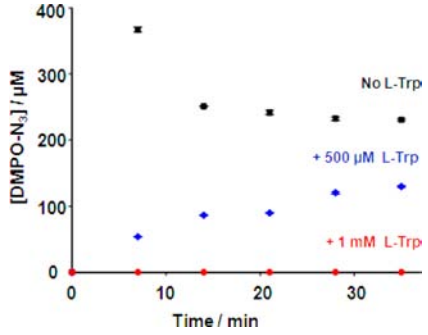

Figure 3. Time dependence of the trapping of azidyl radicals by DMPO (8 mM) in a continuously irradiated solution of **1** (4 mM) in PBS in the absence (black ■) or the presence of 500 μM (blue ◆) or 1 mM (red ●) L-Trp. The light source was a blue LED (λ = 463 nm, 64 mW cm^{-2}).

investigated whether the mechanism of L-Trp suppression of radical trapping proceeds by prevention of photodecomposition of **1** and therefore radical formation or by destruction of azidyl radicals once formed.

We investigated the interaction of L-Trp with **1** and photoactivated **1** by both UV−vis and NMR spectroscopy. The UV−vis absorption spectrum of a solution of **1** (50 μM) after irradiation with blue light (463 nm, 64 mW cm^{-2}) for 30 min in the absence or presence of various amounts of L-Trp showed a decrease in intensity of the azide-to-Pt^{IV} charge-transfer band at ca. 294 nm (Figure S3), consistent with the photodecomposition of **1** and loss of the Pt^{IV}−N_3 bonds.[7] This suggests that L-Trp does not prevent photodecomposition but destroys the azidyl radicals as or after they are released. The ^{1}H NMR peaks for L-Trp (1 mM) decreased in intensity in the spectrum of a phosphate-buffered D_2O solution (pH* 7.4) containing **1** (4 mM) and DMPO (8 mM) after irradiation with blue light (463 nm, 64 mW cm^{-2}) for times of up to 30 min (Figure S4). The ^{1}H NMR peaks for DMPO remained unchanged, confirming that no azidyl radicals were trapped in the presence of 1 mM L-Trp. In contrast, new peaks corresponding to platinum photoproducts from **1** appeared, similar to those in the absence of L-Trp. Thus, the course of the photodecomposition at the Pt center appears to be similar in the absence and presence of Trp.

Our findings with azidyl radicals are compatible with the data reported by Solar et al.,[21] who showed that azidyl radicals generated by pulse radiolysis can oxidize L-Trp to give the

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radical cation $TrpH^{\bullet+}$, which deprotonates to give the neutral $Trp^{\bullet}$ radical, a process which is energetically favored at pH 7.[19−22] Solar et al.[21] found that one-electron oxidation of L-Trp is the primary reaction of azidyl radicals in solution, so we focused our attention on the detection of free azide as a photoproduct at pH 7. The formation of free azide was confirmed by the ^{14}N NMR spectrum of a phosphate-buffered D_2O solution (pH* 7.4) containing **1** (9 mM), DMPO (18 mM), and 2 molar equiv of L-Trp after irradiation with blue light (463 nm, 64 mW cm^{-2}) for 30 min (Figure S5A), which contained peaks at 228.6 and 77.3 ppm assignable to the terminal nitrogen atom (overlapped with Nβ of the coordinated azide) and the central nitrogen atom, respectively, of N_3^-. The peak at 77.3 ppm was not observed after irradiation of a similar solution in the absence of L-Trp (Figure S5B).

Next, we investigated whether the production of azidyl radicals could be controlled by the presence of L-Trp in cancer cells treated with complex **1**. Neither complex **1** alone nor **1** in the presence of tryptophan had any observable effects on A2780 human ovarian cancer cells in the dark under the experimental conditions used. Also, blue light itself (λ_{max} = 420 nm, 5 J cm^{-2}) had no effect on cell growth (cell viability of 115.7 ± 9.4%). It can be seen from Figure 4A that co-

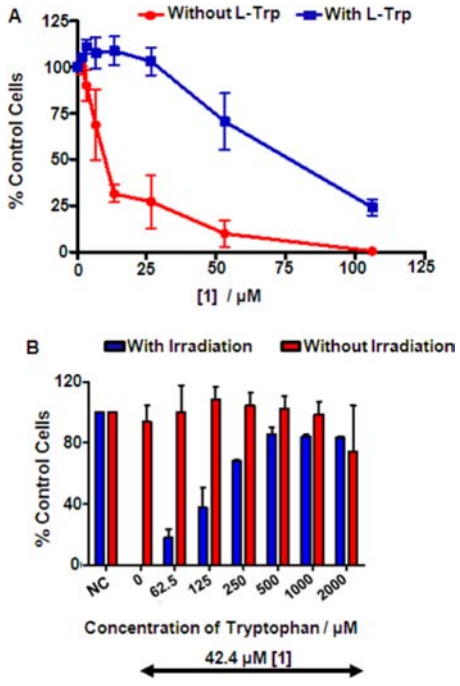

Figure 4. (A) Effect of 500 μM L-Trp on the ability of photoactivated **1** to reduce the viability of A2780 human ovarian cancer cells. Data represent means ± standard errors of the mean for three independent experiments performed in triplicate. Visible-light irradiation (λ_{max} = 420 nm, 5 J cm^{-2}) was used. (B) Effect of varying the L-Trp concentration with 42.4 μM **1** (n = 2 independent experiments). NC: negative control (no L-Trp or complex **1**).

incubation of cells with L-Trp reduced cell death due to **1** following irradiation with visible light relative to incubation with **1** alone. The presence of 500 μM L-Trp conferred a protection factor of ca. 7 based on IC_{50} values (Table 1). Reducing the concentration of L-Trp to approximately that of **1** (ca. 50 μM) still conferred some photoprotection (Figure 4B).

Table 1. Effect of 500 μM L-Trp on the Ability of Photoactivated 1 To Reduce the Viability of A2780 Ovarian Cancer Cells (As Determined by Inhibition of Neutral Red Dye Uptake)

treatment	IC_{50} (μM)[a]	95% interval (μM)	confidence R^2
no L-Trp	8.3	3.4−20.4	0.85
+500 μM L-Trp	59.4	34.7−101.8	0.86

[a]IC_{50} = concentration of **1** that inhibited uptake of the dye by 50%. Data represent the means of three independent experiments

The accumulation of Pt in the cells was not affected by the presence of L-Trp (see Figure S6).

The effect of 500 μM L-Trp on A2780 cancer cells was clearly seen morphologically. In the presence of 500 μM L-Trp, the neutral red dye was taken up by the cells 24 h after exposure to complex **1** and irradiation, indicating viability, whereas few viable cells could be seen in the absence of L-Trp (Figure 5).

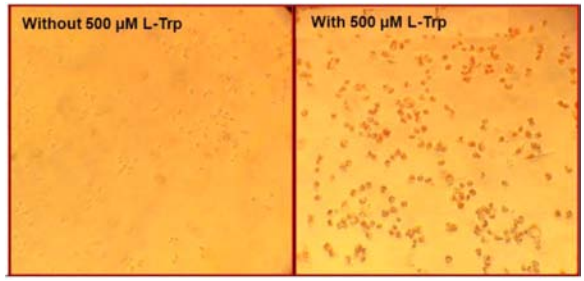

Figure 5. A2780 cells treated with 42.4 μM **1** (left) in the absence and (right) in the presence of 500 μM L-Trp 24 h after irradiation with blue light (λ_{max} = 420 nm, 5 J cm^{-2}). The clearly visible intracellular red staining in the right-hand image is neutral red dye (Leica DMIL microscope, 200× magnification).

These cell data suggest that radical-based mechanisms are important for the photocytotoxicity of complex **1**. Diazido complexes such as **1** may therefore have a dual mechanism of action involving both radical- and platinum-based attack, explaining their high potency and their apparent apoptosis-independent mechanism of action.[23]

Oxidative attack of azidyl radicals on L-Trp residues in proteins might have important consequences for the cellular metabolism of **1** in cancer cells, especially when the Trp residue is part of an electron transport pathway. Interestingly, reduction of N_3 (formed by pulse radiolysis) by the dipeptide TrpTyr has been reported to lead to the formation of $Trp^{\bullet}$ radicals followed by oxidation of the Tyr residue by $Trp^{\bullet}$ to yield $Tyr^{\bullet}$ radicals.[20] In the present case, the nature of the attack sites may depend on initial recognition of Pt^{IV} azido complex **1** and differ from those of azidyl radicals generated by other methods.

Our findings have potential significance for the clinical use of complex **1** in photochemotherapy. The photodecomposition pathways for complex **1** in tumor cells and other tissues should be switchable by raising or lowering the level of L-Trp or L-Trp derivatives such as peptides. Side effects from post-treatment exposure to sunlight, which are a concern in phototherapy, might be ameliorated in the case of **1** by postadministration of L-Trp or a related indole derivative.

Interestingly, the serum L-Trp level of some cancer patients (including breast, lung, and ovarian) is depleted relative to normal controls. This is thought to be due to the activity of indoleamine 2,3-dioxygenase, which converts it to kynuren-

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ine.[24a,b] It is possible that if L-Trp is depleted in cancer patients, and if this is a general finding, then such depletion may aid the effectiveness of **1** in photochemotherapy.

■ ASSOCIATED CONTENT

Supporting Information

Experimental material and procedures; UV−vis, NMR, and EPR spectroscopic data; data tables; and cell work. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare the following competing financial interest(s): P.J. Sadler has ownership interest by patent application GB0120618.

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