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A Photo-Caged Platinum(II) Complex That Increases Cytotoxicity upon Light Activation

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A novel platinum(II) photocaged complex called [Pt(cage)] has been prepared and characterized by X-ray crystallography. The complex contains a photolabile nitrophenyl group incorporated into the backbone of a tetradentate ligand that contains two pyridyl and two amide nitrogen donor sites. The intact complex is unreactive toward ligand-exchange reactions until activation with UV light cleaves the ligand backbone, releasing a Pt^{II} complex that more readily exchanges its ligands, as verified by reaction with a methionine-containing peptide. [Pt(cage)] is non-toxic to MCF-7 cells in the dark, whereas brief UV exposure induces cell death of human breast cancer MCF-7 cells at a level approaching that of cisplatin. By using light to alter the coordination chemistry around the metal center, [Pt(cage)] represents a new strategy for potentially delivering metal-based drugs in a site and time specific manner.

Introduction

Since the initial discovery of cisplatin's cytotoxicity by Rosenberg in the 1960s, platinum-based drugs, which now include carboplatin and oxaliplatin as well as cisplatin, have become a cornerstone of modern anticancer chemotherapy regimens.[1] The clinical effectiveness of these agents, unfortunately, is restricted by dose-limiting toxicity and intrinsic or acquired resistance of some tumors. Significant efforts to understand the cellular response and tumor resistance mechanisms of platinum drugs[2,3] have inspired the development of new compounds to overcome these limitations. Strategies include altering the coordinating ligands on platinum, designing drug delivery systems that distribute platinum compounds selectively to tumor cells, and developing prodrugs that release cytotoxic agents following an activation step.[4,5] In this area, substitution-inert Pt^{IV} agents that are reduced intracellularly to active Pt^{II} compounds are attractive for diminishing off-target cytotoxicity and resistance.[5] More recently, Sadler and co-workers introduced photoactive Pt^{IV} prodrugs in which the reduction occurs only upon illumination with UV light.[6–8]

Photoactive compounds that can deliver cytotoxic drugs intracellularly only at the irradiated site can potentially increase the specificity of a drug and thereby minimize its toxicity to surrounding healthy tissue.[9,10] Such a strategy is envisioned for surface-associated diseases or for internal tissue accessible via endoscopic fiber optic technology.[9] A drawback of the Pt^{IV} complexes reported to date is their sluggish photoactivation, which can take upwards of an hour of UV irradiation.

As an alternative to photoactive Pt^{IV} compounds, we present here a novel photo-caged Pt^{II} complex in which a photoactive nitrophenyl group is incorporated into the ligand backbone. The tetradentate chelating ligand suppresses ligand-exchange reactions to provide a nontoxic Pt^{II} complex, [Pt(cage)]. Activation with light induces bond cleavage of the ligand, as shown in Scheme 1, which converts neutral [Pt(cage)] into a charged and exchange labile Pt^{II} complex **3**. Both properties may be beneficial for cellular retention of the photolyzed compound. Importantly, photoactivation occurs within minutes and induces significant photo-dependent cytoxicity.

Photoactive nitrophenyl groups have been used in countless examples to block (i.e. "cage") the biological activity of a variety of molecules such that exposure to UV light turns on a biological function.[11,12] This strategy is also being explored to develop complexes that allow photoinitiated drug release for applications in photochemotherapy.[10,13–16] The concept of light-activated caged metal ions was first introduced for Ca^{2+},[17] and has only recently been expanded to biologically important d-block metal ions like Cu^{2+} and Zn^{2+}.[18–20] Metal complexes themselves have also been exploited as caging groups, for example ruthenium polypyridines that release bioactive compounds by light-induced ligand dissociation.[21,22] In these contexts, the terminology "cage" implies photocage, where light is used to effect a molecular change that alters a biological response. It does not imply a geometric configuration and is in fact distinct from the classical inorganic definition of cage, which refers to a polycyclic compound having the shape of a cage,

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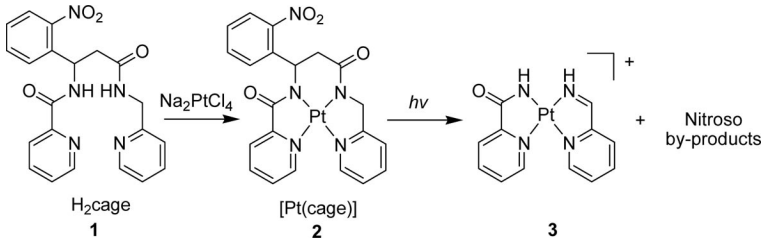

Scheme 1.

or an inclusion compound. The report here demonstrates that a photoactive ligand can be used to unleash the cytotoxicity of a metal-based agent.

Results and Discussion

[Pt(cage)] was prepared in one step by combining equimolar quantities of H_2cage[18] and Na_2PtCl_4 in basic ethanol. Recrystallization from acetone/H_2O permitted analysis by X-ray crystallography that confirmed that two deprotonated amide nitrogens and two pyridyl nitrogens coordinate the Pt^{II} center in square-planar geometry, as shown in Figure 1. In comparison, the copper complex of this ligand, [Cu(OH_2)(cage)], has distorted trigonal bipyramidal geometry that can be attributed to a steric interaction between the α hydrogens on the pyridyl rings.[18] The increased radius of Pt^{II} compared to Cu^{II} alleviates this steric clash to accommodate the square planar arrangement preferred by this d^8 metal ion. The average Pt–amide bond length of 1.987 Å and Pt–pyridyl distance of 2.036 Å are similar to those of [Cu(OH_2)(cage)], 1.943 Å and 2.034 Å respectively.

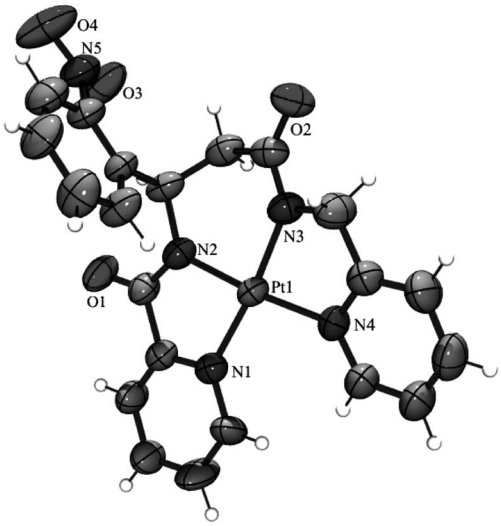

Figure 1. ORTEP plot of [Pt(cage)] showing 50% thermal ellipsoids. Selected bond lengths and angles: Pt–N1, 2.036(6); Pt–N2, 1.979(6); Pt–N3, 1.994(7); Pt–N4, 2.036(6) Å; N2–Pt–N3, 93.7(3); N2–Pt–N1, 79.8(3); N3–Pt–N1, 173.4(3); N2–Pt–N4, 173.7(3); N3–Pt–N4, 80.2(3); N1–Pt–N4, 106.4(3)°.

To investigate the photoreactivity of [Pt(cage)], solutions of the complex dissolved in pH 7.4 phosphate buffer were irradiated in a Rayonet photoreactor and monitored by UV/Vis spectrophotometry. As shown in Figure 2, spectral changes are apparent within seconds of illumination and exhibit an increase at 320 nm that is characteristic of a nitroso photoproduct. These data suggest that the photoreaction and in turn cleavage of the ligand backbone is complete in approximately 2 min. Analysis of the reaction mixture by liquid chromatography-mass spectrometry (LC-MS) revealed **3** as the major product (see Supporting Information), implying that ligand cleavage occurs at two sites. The products are slightly different from those observed for the apo-ligand or its Cu^{II} complex, where the ligand is cleaved only at one position.[18] We hypothesize that upon excitation of the nitrophenyl group, initial bond cleavage occurs to release the picolinamide fragment shown coordinated to Pt in **3** and a nitroso photoproduct that subsequently undergoes a Norrish type II photoreaction to liberate the imine fragment bound to Pt in compound **3** along with nitroso-containing by-products (see Supporting Information). The quantum efficiency for the photolysis of [Pt(cage)] was determined to be 0.75 (Supporting Information). The ligand itself has a quantum efficiency of 0.73, which indicates that coordination by Pt^{II} does not decrease photolysis efficiency, as previously observed for [Cu(OH_2)(cage)].[18]

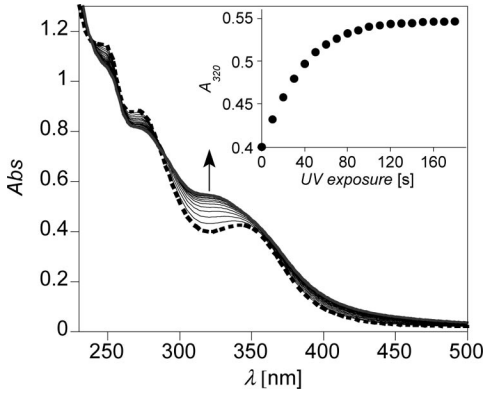

Figure 2. UV/Vis spectra of 80 μM [Pt(cage)] in phosphate buffer at pH 7.4 photolyzed in 10 s intervals for a total of 180 s (initial spectrum at time zero is dashed line); inset: absorbance at 320 nm vs. irradiation time shows that the compound is completely photolyzed within 2 min.

The photolysis experiments described above indicate that two bidentate photoproducts remain coordinated to Pt^{II} following illumination. In order to determine whether such a product could induce cell death, we treated human breast carcinoma MCF-7 cells with [Pt(cage)] and monitored cyto-

toxicity of irradiated vs. non-irradiated samples. For comparison, cells were also treated with cisplatin. As shown in Figure 3, control cells exposed to 2 min of UV light remain viable over the course of 96 h and display no increase in cell death compared to cells kept in the dark. As expected, cisplatin induces cytotoxicity in a dose-dependent manner with concentrations ranging from 50–200 μM. Exposure of cisplatin-treated cells to UV light only subtly increases sensitivity of the cells to cisplatin cytotoxicity. On the other hand, cells treated with up to 200 μM [Pt(cage)] and left in the dark remain viable over the 96 h timecourse, whereas those that are also exposed to 2 min of UV irradiation show a significant increase in cell death. These results suggest that intact [Pt(cage)] itself is non-toxic, but activation with UV-light releases photoproducts that induce cell death in a light-dependent fashion.

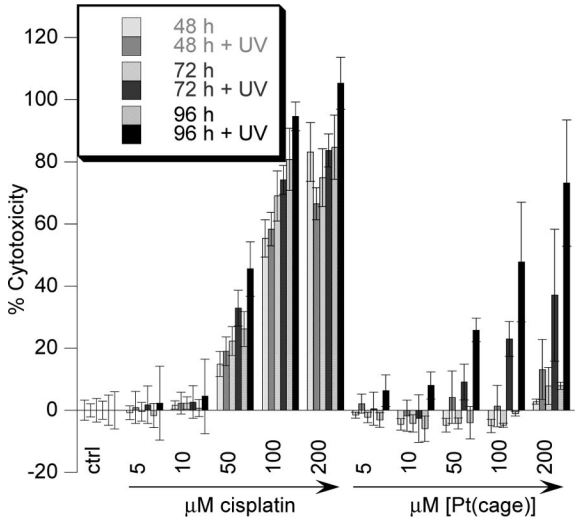

Figure 3. Results of cytotoxicity assay performed on MCF-7, human breast carcinoma cell line. Cells were treated with 5–200 μM cisplatin or [Pt(cage)] and either left in the dark or exposed to UV light for 2 min (+ UV). Cell death was assessed by the LDH release assay after 48, 72, or 96 h, as indicated. Control cells (ctrl) received no drug treatment but were exposed to UV light.

Control experiments using H_2cage show that the ligand itself is cytotoxic, even in the dark. As shown in Figure 4, a 200-μM dose of H_2cage causes nearly 60% cell death within 48 h, even in the absence of UV irradiation. Cytotoxicity further increases when the cells also receive 2 min of UV exposure, even at the earliest timepoint monitored in these experiments (48 h). In contrast, cells treated with 200 μM [Pt(cage)] and irradiation remain mostly viable at 48 h, showing less than 15% cell death. The combined results shown in Figure 3 and Figure 4 indicate that coordination to Pt^{II} actually mitigates an inherent cytotoxicity of the ligand. Furthermore, the timing discrepancy in light-activated cell killing implies different mechanisms of toxicity for [Pt(cage)] and H_2cage, which photolytically decompose into different organic fragments. The observations that the carrier ligand's toxicity is increased by light-activation but can be masked by metal binding suggests a possible synergy between the metal component and ligand component that could be further exploited to improve light-activated cell killing.

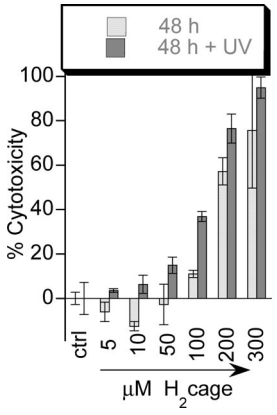

Figure 4. Results of LDH cytotoxicity assay performed on MCF-7 cells treated with 5–300 μM H_2cage and either left in the dark or exposed to UV light for 2 min prior to 48 h incubation.

The binding of platinum drugs to DNA is believed to be the primary biological interaction responsible for their anticancer properties.[2] In an attempt to visualize such interactions for [Pt(cage)] or its photoproducts, we used agarose gel electrophoresis. Intact [Pt(cage)] at concentrations up to 300 μM had no effect on the migration of either circular or linear plasmid DNA through the gel, suggesting that the compound does not react with DNA to form platinum adducts, at least under the conditions tested (see Supporting Information). This result was expected, given the stability of the tetradentate chelator designed to minimize ligand exchange reactions. However, photolyzed samples of [Pt(cage)] also failed to cause a shift in DNA migration. The lack of evidence for light-dependent DNA platination requires further investigation in order to understand the biological activity of **3** that induces the cytotoxicity observed in Figure 3. Notably, other tetraamine Pt^{II} complexes have shown cytotoxicity in cancer cell lines.[23]

Pt^{II} interactions with sulfur-containing biomolecules are believed to play various roles in the uptake, excretion, resistance and toxicity of platinum drugs.[24] Current Pt-based drugs are thought to enter cells by a combination of passive diffusion and facilitated uptake by transporters that include the sulfur-rich copper transport protein Ctr1. We, and others, have shown that the methionine-rich extracellular regions of Ctr1 are capable of coordinating Pt drugs and, especially in the case of cisplatin, inducing complete loss of the carrier ligands.[25–27] Because such interactions are likely to diminish the cytotoxic potential of Pt drugs, we were curious to compare the difference in reactivity of [Pt(cage)] pre- and post-photolysis with a model Ctr1 peptide. To show that photolysis of [Pt(cage)] causes a change in its reactivity we monitored the ability of the complex to react with the peptide AcMMMMPMTFK that we have previously shown reacts with cisplatin, carboplatin, and oxaliplatin.[25] As shown by the LC/MS data in Figure 5, intact [Pt(cage)] shows no interaction with AcMMMMPMTFK

even after 24 h of incubation at 37 °C. On the other hand, photolyzed samples show a decrease in signal intensity for both **3** {compared to photolyzed [Pt(cage)] samples without peptide} and the peptide (see Supporting Information, Figure S4), suggesting that a reaction has occurred. Furthermore, new peaks emerge in the UV chromatogram that indicate a complex mixture of products. While most of these species were not able to be identified, one provided a strong ion peak in the mass spectrum at *m*/*z* 1382.5, which is consistent with formation of $[Pt(AcMMMMPMTFK)]^+$. This result further confirms that the intact [Pt(cage)] is inert to ligand subsitution, whereas the photoproduct can react with biomolecules and shed the bidentate ligands.

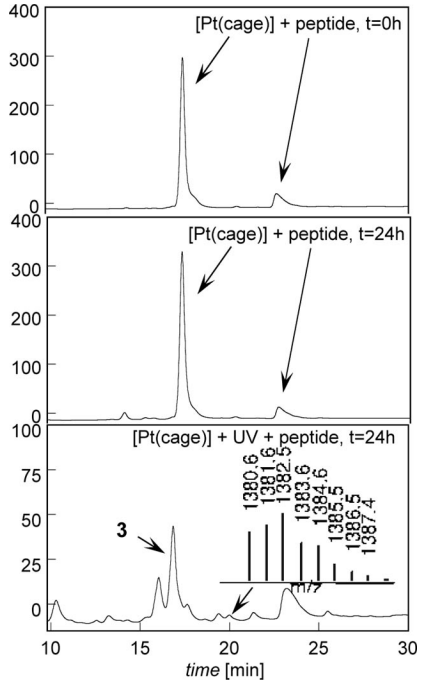

Figure 5. Chromatography traces for reaction mixtures of [Pt(cage)] and peptide AcMMMMPMTFK incubated at 37 °C with or without photolysis. Top: trace recorded just after mixing at time zero. Middle: 24 h sample that was not photolyzed shows no changes. Bottom: the UV trace for photoproduct **3** elutes at the same time as [Pt(cage)], but is identified by its mass spectrum (see Supporting Information). Inset: mass spectrum corresponding to elution time 19–21 min, identified as $[Pt(AcMMMMPMTFK)]^+$. For all traces: the peptide absorbs weakly at 254 nm, so provides a weak intensity signal compared to [Pt(cage)]. See Supporting Information for analysis at 228 nm, where the peptide intensity is greater.

The lack of interaction between the methionine-rich peptide and [Pt(cage)] implies that, prior to photo treatment, [Pt(cage)] is unlikely to enter cells via a Ctr1-mediated pathway or be stripped of its carrier ligand. [Pt(cage)] is a neutral complex with a molecular weight less than 500 g/mol, which may be favorable for passive diffusion into cells. Like cisplatin, the [Pt(cage)] photoproduct **3** is a charged complex, which makes it less likely to diffuse through biological membranes and suggests that it could become trapped in the cell to facilitate its cytotoxic effects. This change from a neutral to charged complex may be another beneficial property imparted by the photoactivation of [Pt(cage)] in addition to the tetradentate-to-bidentate conversion.

Conclusions

In conclusion, we have presented an inert Pt^{II} compound that upon irradiation with UV light uncages a biologically active Pt^{II} complex by cleavage of the ligand backbone. [Pt(cage)] was shown to be non-toxic to human breast carcinoma MCF-7 cells in the dark, however upon irradiation its cytotoxicity increased by 65%. [Pt(cage)] will be a valuable tool for delivering Pt intracellularly in a site and time specific manner and represents an alternative strategy for activating metal-based drugs with light.

Experimental Section

CCDC-756957 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see also the footnote on the first page of this article): Experimental procedures.

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