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Modulation of ligand fluorescence by the Pt(II)/Pt(IV) redox couple

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Dedicated to Professor Jon Zubieta on the occasion of his 65th birthday.

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ABSTRACT

The dangling carboxylic acid moiety of the known platinum(II) complex, [Pt(edma)Cl_2] (edma = ethylenediaminemonoacetic acid), was functionalized via amide coupling chemistry with benzyl amine and dansyl ethylenediamine to afford the derivatives [Pt(edBz)Cl_2] (**1**) and [Pt(edDs)Cl_2] (**2**). Subsequent oxidation of these platinum(II) complexes with iodobenzene dichloride in DMF yielded the respective platinum(IV) analogs, [Pt(edBz)Cl_4] (**3**) and [Pt(edDs)Cl_4] (**4**). All four platinum complexes were characterized by multinuclear NMR spectroscopy, IR spectroscopy, electrospray ionization mass spectrometry, and elemental analysis. In addition, compounds **1** and **3** were structurally characterized by X-ray crystallography. The photophysical properties of the compounds bearing the fluorescent dansyl moiety, **2** and **4**, were evaluated. The emission quantum yields of **2** and **4** in DMF are 27% and 1.6%, respectively. This large difference in emission efficiency indicates that the platinum(IV) center in **4** is more effective at quenching the dansyl-based fluorescence than the platinum(II) center in **2**. Time-dependent density functional theory calculations indicate that **4** has several low-lying singlet excited states that energetically lie below the primary radiation-accessible excited state of the dansyl fluorophore. These low-energy excited states may offer non-radiative decay pathways that lower the overall emission quantum yield. Treatment of **4** with biologically relevant reducing agents in pH 7.4 phosphate-buffered saline induces a 6.3-fold increase in emission intensity. These results demonstrate that **4** and future derivatives thereof may be useful for imaging the reduction of platinum(IV) complexes in living systems, chemistry of importance for evolving platinum-based anticancer drug strategies.

1. Introduction

Platinum-based drugs are effective agents for a number of different cancers [1,2]. Dose-limiting toxic side effects and acquired resistance, however, limit the broader applicability of this class of compounds. The search for new platinum anticancer agents with fewer such drawbacks is an active area of research [3,4]. Among the new classes of platinum compounds explored, octahedral platinum complexes in the +4 oxidation state show considerable promise. Platinum(IV) anticancer complexes have several advantages over first- and second-generation square-planar platinum(II) analogs [5–7]. The additional two ligand-binding sites enable synthetic modification and fine tuning of the pharmacokinetic parameters. Compared to platinum(II) complexes, however, the mechanism of action of platinum(IV) complexes is less well understood. In most cases, these complexes are best reduced first by two electrons to form square-planar platinum(II) complexes in a process that facilitates binding to proteins, DNA, or other cellular targets [8]. Nevertheless, questions remain about this reductive activation step. It is not clear how quickly, by what means, and where such reduction occurs in living systems. X-ray fluorescence and absorption spectroscopy [9–11] has been used to address these questions. These methodologies, however, are not applicable to live cells and generally require synchrotron radiation.

Optical fluorescence microscopy for imaging analytes and processes in living systems is well established [12]. This method has been used to study the cellular localization and uptake of platinum–fluorophore conjugates, which were designed as models for related platinum anticancer drugs [13–15]. Although fluorescence microscopy, unlike X-ray techniques, cannot directly provide information about the oxidation state of metal ions, it is possible to apply ligand systems in which the fluorescence intensity is modulated depending on the oxidation state of the coordinated metal [16,17]. Such a system involving the Pt(IV)/Pt(II) redox couple has recently been described [18], in which analogous platinum(II) and platinum(IV) complexes were coordinated to fluorescent coumarin ligands by means of an aniline functional group on the dye. The authors reported a 7-fold greater emission intensity for the platinum(II) compared to the platinum(IV) complex in DMF solution. Furthermore, confocal fluorescence microscopic imaging analyses revealed strong localization of the complexes in the cytosol and lysosomes. The free coumarin ligands exhibited cellular localization similar to that of the complexes,

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thus raising the possibility that the coumarin ligands are displaced from platinum in the cell. Similar systems with strongly coordinated bidentate ligands may be able to provide information about platinum(IV) reduction in live cells.

In the present article we describe our work to design a platinum–fluorophore conjugate that can be used to monitor platinum(IV) reduction in living systems by an emissive turn-on response. We present the synthesis and characterization of the dansyl fluorophore-bearing platinum(II) and platinum(IV) complexes, **2** and **4**, as well as non-fluorescent analogs **1** and **3** (Scheme 1). Photophysical studies of **2** and **4** indicate the platinum(IV) complex to have a significantly lower emission quantum yield than the platinum(II) complex; upon reduction of **4**, a 6.3-fold emission turn-on response is observed in aqueous buffer. Additionally, computational studies are reported that rationalize the increased fluorescence quenching of **4**.

2. Experimental

2.1. General considerations

All reactions were carried out under normal atmospheric conditions. Solvents were used as received without additional drying or purification. The compounds, [Pt(edma)Cl_2] (edma = ethylenediaminemonoacetic acid) [19], dansyl ethylenediamine (Ds-en) [20], and iodobenzene dichloride [21], were synthesized as previously described. Benzylamine and carbonyldiimidazole (CDI) were purchased from Sigma–Aldrich and used as received.

2.2. Physical measurements

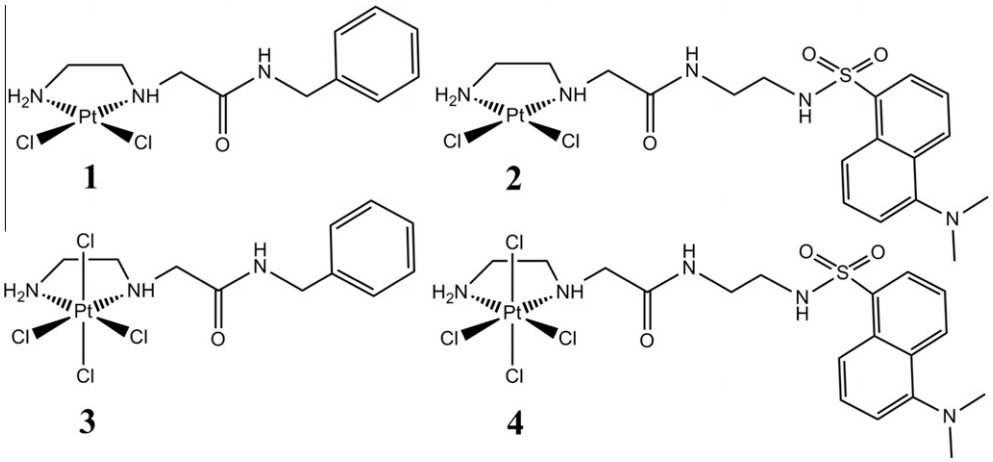
NMR spectra were recorded on a Bruker DPX-400 spectrometer in the MIT Department of Chemistry Instrumentation Facility at 20 °C. ^{1}H and ^{13}C{^{1}H} NMR spectra were referenced internally to residual solvent peaks, and chemical shifts are expressed relative to tetramethylsilane, $SiMe_4$ (δ = 0 ppm). ^{195}Pt{^{1}H} NMR spectra were referenced externally to K_2PtCl_4 in D_2O (δ = −1628 ppm). NMR spectra of all compounds are shown in the Supplementary data (Figs. S1–S22). Fourier transform infrared (FTIR) spectra were recorded with a ThermoNicolet Avatar 360 spectrometer running the OMNIC software. Samples were prepared as KBr disks. Cyclic voltammograms were obtained at room temperature using a VersaSTAT3 potentiostat from Princeton Applied Research accompanied by the V3 Studio software. A three-electrode system was used, comprising a glassy carbon working electrode, a Pt wire auxiliary electrode, and a Ag/AgCl (aqueous saturated NaCl) reference electrode. Samples were prepared as 2 mM solutions in DMF with 0.1 M (Bu_4N)(PF_6) as the supporting electrolyte. Peak potentials are reported at a scan rate of 100 mV/s. The ferrocene/ferrocenium redox couple was 0.54–0.55 V versus Ag/AgCl using the setup described here. Optical absorption spectra were recorded with a Cary 1E spectrophotometer. Emission spectra were obtained with a Photon Technology International QM-4/2003 fluorimeter. Quantum yields for fluorescence were measured using quinine sulfate in 0.1 M H_2SO_4 (Φ = 0.58) as the reference [22] over a range of at least five different absorbance values. For measuring these values in phosphate buffered saline (PBS), the samples were diluted from DMF solutions to give aqueous solutions containing less than or equal to 1% DMF. For all photophysical measurements, the sample temperature was maintained at 25.0 ± 0.5 °C using a circulating water bath.

2.3. Synthesis of [Pt(edBz)Cl_2] (**1**)

A solution of CDI (0.230 g, 1.42 mmol) in 10 mL of DMF was added to a solution of [Pt(edma)Cl_2] (0.535 g, 1.39 mmol) in 10 mL of DMF. The resulting mixture was heated at 60 °C for 10 min, and then sparged with N_2 for 5 min. Benzylamine (0.152 g, 1.42 mmol) in 15 mL of DMF was added dropwise to the solution containing the activated platinum complex. After stirring for 12 h, the solution was concentrated to 10 mL under reduced pressure and elevated temperature (60 °C) and then filtered through Celite. The addition of 10 mL of water afforded the desired compound as a pale yellow solid, which was isolated by filtration and washed sequentially with 5 mL of water, 2 × 5 mL of ethanol, and 2 × 5 mL of diethyl ether before being dried in vacuo. Yield: 0.268 g (40%). M.p. 298–300 °C (dec). ^{1}H NMR (400 MHz, DMF-d_7): δ 8.60 (t, 1H), 7.35–7.26 (m, 5H), 6.15 (br s, 1H), 5.48 (br s, 2H), 4.43 (d, 2H), 4.27 (d, 1H), 3.71 (dd, 1H), 3.12–3.07 (br m, 1H), 2.82–2.72 (br m, 2H), 2.57–2.55 (br m, 1H). ^{13}C{^{1}H} NMR (100 MHz, DMF-d_7): δ 168.2, 139.4, 128.6, 127.7, 127.2, 57.8, 55.1, 47.3, 42.8. ^{195}Pt{^{1}H} NMR (86 MHz, DMF-d_7): δ −2339. IR (KBr, cm^{-1}): 3335 m, 3274 s, 3202 m, 2940 w, 1654 vs, 1576 m, 1545 s, 1455 w, 1436 m, 1425 m, 1392 vw, 1279 w, 1243 m, 1168 w, 1116 w, 1091 w, 1041 w, 967 w, 758 m, 702 s, 611 w, 572 w, 525 w. ESI-MS (negative-ion mode): *m/z* 509.4 [M+Cl]$^-$, 944.6 [2M–H]$^-$, 980.9 [2M+Cl]$^-$. *Anal.* Calc. for $C_{11}H_{17}Cl_2N_3OPt$: C, 27.92; H, 3.62; N, 8.88. Found: C, 28.30; H, 3.65; N, 8.85%.

2.4. Synthesis of [Pt(edDs)Cl_2] (**2**)

A solution of CDI (0.206 g, 1.27 mmol) in 20 mL of DMF was added to a solution of [Pt(edma)Cl_2] (0.469 g, 1.21 mmol) in



Scheme 1. Structures of compounds **1**–**4**.