Luminescent cyclometalated platinum(II) complexes with amino acid ligands for protein binding†

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Pt(C^N)(phe) (1, C^N = 2-(2′-thienyl)pyridine, phe = phenylalanine) shows a high binding affinity (*ca.* 10^6 dm^3 mol^{-1}) and selectivity towards human serum albumin (HSA) and such binding is accompanied by an enhancement of photoluminescence at 562 nm; both the protein binding affinity and cytotoxicities of [Pt(C^N)(phe) (1), Pt(C^N)(trp) (2, trp = tryptophan) and Pt(C^N)(gly) (3, gly = glycine)] are affected by the amino acid ligand with 1 having an IC_{50} of up to 1 μM against a number of carcinoma cell lines.

In the context of developing metal-based therapeutics and luminescent probes for biomolecules, it would be useful to introduce binding motif(s) that allow for specific interaction(s) between the metal compound and biomolecules.[1] Specific binding to metal transport proteins such as human serum albumin could lead to accumulation of the metal compound in target cells.[2] In literature, there have been extensive studies on interactions between luminescent metal compounds and DNA;[3,4] related works with plasma proteins remain sparse.[5] We were attracted to the reports on Pt^{II}–amino acid complexes revealing their potential applications as antitumor drug leads due to their DNA binding properties, HMG1 protein affinity for the platinated DNA and cytotoxicity against HeLa cells.[6,7] We envision that the "Pt(C^N)(amino acid)" systems are potentially useful for the luminescent signalling of biomolecules. The amino acid auxiliary ligand could direct the binding or recognition of "Pt(C^N)(amino acid)" towards biomolecules through complementary H-bondings,[8] and the emission of the "Pt(C^N)" moiety could be a probe for such a binding process.[9] Here we show that complex **1** displays a strong binding affinity and selectivity towards HSA and such binding is accompanied by an enhancement of photoluminescence. Comparative studies with **2** and **3** reveal that the HSA binding reaction and *in vitro* cytotoxicities of Pt(C^N)(amino acid) complexes are affected by the amino acid ligand (Fig. 1).

Complexes **1**–**3** do not bind to DNA as revealed by a restriction endonuclease fragmentation assay and a UV melting experiment (see ESI†). However, they bind to HSA in a buffer solution. The absorption spectra of **1** treated with different concentrations of HSA in phosphate buffered saline (PBS) reveal spectral changes with hypochromism (11%) at 395 nm and about 10 nm of bathochromic spectral shift (see ESI†). Plotting r/C_F against r according to the Scatchard equation (see ESI†),[10] the binding constant K and binding stoichiometry n at 20.0 °C were found to be 1.0×10^6 mol^{-1} dm^3 and 1.1 accordingly. The K and n values for **2** and (**3** in parentheses) were similarly determined to be 7.94×10^5 (1.51×10^5) mol^{-1} dm^3 and 0.6 (1.2) respectively.

Complex **1** is weakly emissive in PBS buffer solution. However, in the presence of HSA, **1** exhibits an intense emission at λ_{max} = 562 nm, the intensity of which reaches a saturation level at [HSA]/[Pt] ⩾ 3 (Fig. 2). As depicted by the plot of I/I_o *vs.* [HSA]/[**1**] (I and I_o are emission intensities with and without HSA), *up to 10-fold intensity enhancement* was observed at [HSA] : [**1**]

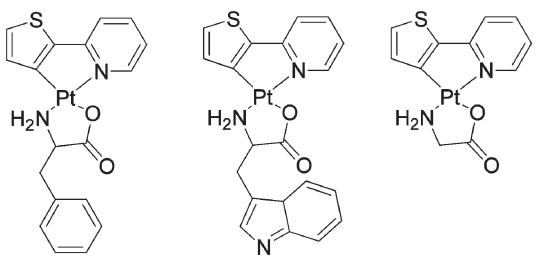

Fig. 1 Structures of the platinum(II) complexes with the amino acid ligand.

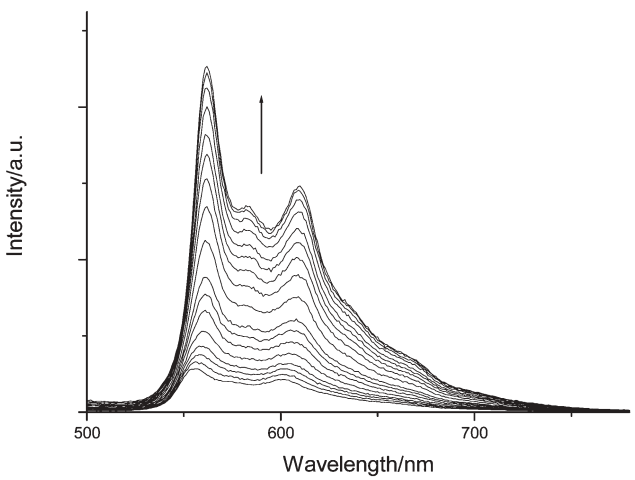

Fig. 2 Emission spectral traces of **1** (4.5 μM) in PBS buffer with increasing ratio of [HSA]/[Pt] = 0–3.0 at 20.0 °C.

† Electronic supplementary information (ESI) available: experimental details; synthesis and characterisation of complexes **1-3**; restriction endonuclease fragmentation assay (Fig. S1); UV melting curve (Fig. S2); absorption titration spectra (Fig. S3); emission titration spectra (Figs. S4–S6); chromatogram (Fig. S7); flow cytometric analysis data (Fig. S8); emission intensity data (Table S1); native polyacrylamide gel electrophoresis data (Table S2). See http://www.rsc.org/suppdata/cc/b4/b414936j/

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ratio $\geqslant$ 3 : 1 (see ESI†). As a control study, no enhancement in emission intensity was observed upon addition of cysteine [cysteine : Pt = 10 : 1] to a PBS buffer solution of **1**. For **2** and **3**, the emission intensity increased by less than 5-fold upon addition of HSA (see ESI†). Thus, the best result was obtained for **1**, which displays a "molecular light-switch" effect upon binding to HSA.

The binding preference of **1** to different components of plasma proteins [α-human globulins (α-HG), β-human globulins (β-HG) and γ-human globulins (γ-HG)] was evaluated by competitive emission study. The complex displays a strong preference for HSA binding (see ESI†) as evidenced by a less than 17% change in the emission intensity upon addition of γ-HG or (α, β and γ-HG) to a HSA–Pt (bound form of HSA and Pt^{II}–amino acid complex) solution at normal or hypoalbuminemia ratios.[11] Here, hypoalbuminemia refers to a low level of albumin in the blood which could be an indication of viral hepatitis, malnutrition, carcinomatosis or severe infections.

The binding preference to HSA was further examined by native polyacrylamide gel electrophoresis (nu-PAGE). The electrophoresis results of HSA at different plasma protein ratios in the absence and presence of **1** are depicted in Fig. 3. Incubating pure HSA (15 μM) in PBS buffer (10 mM PBS, 10 mM Na_2HPO_4–NaH_2PO_4, 0.9% NaCl, pH 7.4) did not show any bands under long UV lamination based on a gel electrophoresis assay (lane A in Fig. 3a). Addition of **1** (15 μM) to a pure HSA solution resulted in the appearance of two bands, which correspond to the monomeric (M1: 80.9%) and dimeric (D1: 19.1%) HSA structures (lane B in Fig. 3a). These bands were also observed in the absence of **1** upon coomassie blue staining (lane A in Fig. 3b). These results indicate that the HSA monomer and HSA dimer could be detected under long UV lamination in the presence of **1**, due to the molecular light-switch effect upon binding of **1** to HSA.

Treatment of a mixture of plasma proteins (HSA, α, β and γ-HG) at normal or hypoalbuminemia ratios with **1** resulted in the appearance of three bands, which correspond to the HSA monomer (M), HSA dimer (D) and a mixture of plasma proteins (P) (lanes D and F in Fig. 3a). There was less than a 5% change in the emission intensity of the bound HSA–Pt upon addition of α, β and γ-HG protein competitors at normal or hypoalbuminemia ratios (see ESI†), thus supporting the claim that **1** has a strong preference for binding to HSA.

Anion-exchange fast protein liquid chromatography (FPLC) also revealed the general HSA preference. The chromatograms of the solution obtained by incubating HSA at different plasma protein ratios in the presence of **1** (0.37 mM) are given in the ESI. HSA and other plasma proteins (α, β and γ-HG) can be separated by the anion-exchange column. HSA and derivatives including HSA–Pt and free HSA were eluted out between 15–17 min while γ-HG and its γ-HG–Pt were eluted out at ~1 min.[12,13] The

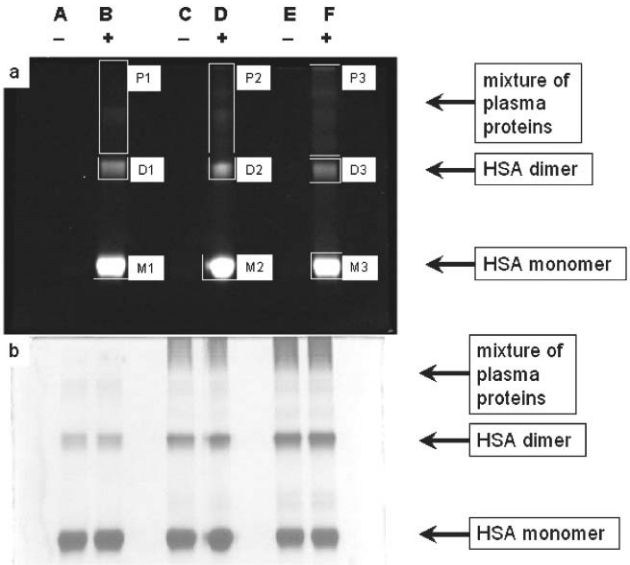

Fig. 3 Nu-PAGE electrophoresis results of HSA at different plasma protein ratios in the absence (–) and presence (+) of **1**. Lanes A and B are pure HSA. Lanes C and D are plasma proteins at the hypoalbuminemia ratio. Lanes E and F are plasma proteins at a normal ratio. (a) Observed under long UV lamination. (b) Observed after coomassie blue staining.

distribution of the Pt^{II} complex (Pt) over different fractions was measured by the emission intensity. Over 95% of the Pt^{II} complex was distributed over the fractions containing HSA. Less than 5% was distributed over the fractions containing α, β and γ-HG.

The cytotoxicities of **1**–**3** against five human carcinoma cell lines HeLa, HepG2, SF-268, NCI-H460, MCF-7 and normal CCD-19Lu were studied; the results are listed in Table 1. The cytotoxicity was measured by a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-tetrazolium bromide] assay, and the IC_{50} values were determined from the dose-dependence of surviving cells after exposure to the platinum(II) complexes for 48 h. The IC_{50} values under our experimental conditions (final concentration $\leqslant$4% DMSO) are in the order: **1** > cisplatin $\cong$ **2** > **3** > free ligands (C^N, phe, trp or gly). Importantly, the decrease in IC_{50} values from **1** to **2** and **3** parallels the corresponding decrease in HSA binding affinity.

Based on flow cytometric analysis, complex **1** induced 41.5 ± 3.3% apoptosis selectively leading to cancer cell death and only 5.4 ± 1.1% necrosis was detected (see ESI†).

In summary, the "Pt(C^N)(amino acid)" system has been demonstrated to bind to protein, and the selectivity of such a binding reaction is dependent upon the amino acid ligand and accompanied by photoluminescence enhancement. This characteristic of the "Pt(C^N)(amino acid)" system suggests that with

Table 1 Cytotoxicities of **1**–**3** and cisplatin in five human carcinoma cell lines, HeLa, HepG2, SF-268, NCI-H460, MCF-7 and normal CCD-19Lu

	IC_{50} [μM]					
Complex	HeLa	HepG2	SF-268	NCI-H460	MCF-7	CCD-19Lu
1	7.4 ± 0.3	8.3 ± 0.8	0.8 ± 0.1	3.9 ± 0.3	1.8 ± 0.2	9.6 ± 0.4
2	45.0 ± 0.5	47.9 ± 1.5	13.3 ± 0.3	27.2 ± 1.4	18.7 ± 1.5	32.0 ± 0.5
3	>100	>100	56.7 ± 2.4	>100	76.9 ± 2.1	>100
Cisplatin	11.6 ± 0.2	20.6 ± 1.9	15.6 ± 0.2	25.1 ± 3.4	19.1 ± 1.7	>100

further modification of the cyclometalated and amino acid ligands, it is possible to develop this class of Pt^{II} compounds as potential diagnostic agents.

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