A Platinium(II)-Based Molecular Light Switch for Proteins

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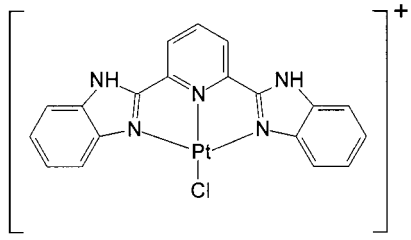
Keywords: Fluorescent probes / Platinum / Proteins / Tridentate ligands

The platinum(II) complex $[Pt(bzimpy)Cl]^+$ (**1**) [bzimpy = 2,6-bis(benzimidazo-2-yl)pyridine] has been synthesized and characterized by FAB mass spectrometry and UV/Vis, NMR, and emission spectroscopy. Complex **1** emits weakly from its ^{3}MLCT state in aqueous solution but strongly in nonaqueous media. Complex **1** exhibits a molecular light switch effect with a drastic enhancement in its emission intensity and a 72-nm blue shift in the emission maxima in the presence of bovine serum albumin (BSA). Denaturation of BSA in the presence of 6 M urea leads to a decrease in the emission intensity of the complex, which suggests its binding to the hydrophobic pockets of the protein. In the presence of hemoglobin, however, quenching of the emission of the complex is observed due to its binding to Cys104 in subunit C and the resultant fluorescence resonance energy transfer from the platinum(II) complex to the heme.

In recent years, there has been continuing interest in designing new molecules for the development of novel chemo- and biosensors. However, most of these studies deal with organic molecules as chemo- and biosensors. In recent times, considerable attention has been paid to the synthesis of inorganic chemosensors.[1] Although metal complexes exhibit luminescent behavior like organic molecules, the utility of these metal complexes as biosensors still remain sparse. In the last decade, Barton et al. have demonstrated that $[Ru(dppz)(bpy)_2]^{2+}$ behaves as a molecular light switch for DNA.[2] Despite extensive work on organic fluorophores such as 8-anilino-1-naphthalenesulfonic acid (ANS) as a probe to study the conformational changes of proteins,[3] no metal complex with a similar behavior has yet been reported.

Among the various transition metal ions, platinum plays a vital role in cancer research. While there are a number of reports on the interaction of platinum(II) complexes with DNA, there are relatively few reports on the binding of platinum(II) complexes to proteins.[4,5] Even though most platinum(II) complexes are nonluminescent at room temperature, a few of them have been exploited as chemosensors.[6] Recently, Grove et al. have discussed the vapochromic properties of $[Pt(Me_2bzimpy)Cl]^+$,[7] and Che et al. have recently demonstrated the use of PEG attached $[Pt(CNN)L]^+$ as a molecular light switch for proteins,[8] although studies pertaining to this complex have been limited to albumins. Very recently, the same group has demonstrated the molecular light switch effect specifically to human serum albumin (HSA) exhibited by a platinum(II) complex containing a cyclometalated ligand anchored to amino acids.[9] In this context, we have developed a new platinum-based biosensor with a tridentate ligand, bzimpy, $[Pt(bzimpy)Cl]^+$ (**1**). To the best of our knowledge, this is the first report of a platinum-based transition metal complex that mimics the behavior of an organic molecule such as ANS.



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Complex **1** was isolated as its PF_6^- salt by refluxing a DMSO solution of bzimpy and K_2PtCl_4 in a 1:1 molar ratio. The absorption spectrum of **1** in DMSO exhibits intense bands at 320 and 380 nm due to an intraligand π–π* transition of the ligand bzimpy. A broad, featureless absorption band at 420 nm has been assigned to a ^{1}MLCT [Pt(5d)–π*(bzimpy)] transition similar to $[Pt(L18)Cl]^+$ [L18 = 2,6-bis(1-octadecylbenzimidazol-2-yl)pyridine].[10] Complex **1** exhibits a very broad and weak ^{3}MLCT emission centered at 625 nm in aqueous solution at room temperature upon excitation at 370 nm. The excited state lifetime of complex **1** is 0.19 μs in Tris buffer solution (pH 7.0) at 298 K. The excited state lifetime of complex **1** is less than that of other platinum(II) complexes such as PtPren and PtPrtmen, whose lifetimes are 4.2 and 3.6 μs, respectively.[11]

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  DOI: 10.1002/ejic.200500335

First, the effect of solvents on the emission spectrum of complex **1** was examined. On decreasing the polarity of the solvent, the emission maximum of the complexes shifts towards lower wavelength from 625 to 560 nm, with an increase in the emission intensity (Figure 1). This type of behavior has previously been observed for organic fluorophores such as ANS, where the emission maximum shifts to lower wavelength on decreasing the polarity of the solvent.[2] In order to investigate the molecular light switch effect of complex **1** in the presence of proteins, we investigated the emission properties of complex **1** with bovine serum albumin (BSA), chicken egg albumin, lysozyme, and hemoglobin. These proteins were chosen as they cover a range of hydrophobicity.

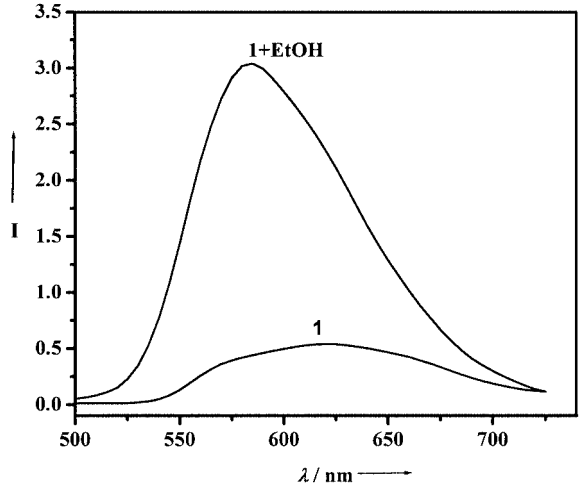

Figure 1. Emission spectra of **1** (50 μM) in the absence and presence of EtOH (λ_{ex} = 370 nm; 298 K; pH 7.0).

Interestingly, on addition of BSA (5 μM), the emission intensity of complex **1** (10 μM) increases 70-fold, with a drastic blue-shift of 72 nm in the emission maximum from 625 nm to 553 nm; the emission band gets sharper on increasing the amount of BSA (Figure 2). A similar behavior of the emission of complex **1** was observed in the presence of chicken egg albumin also. This enhancement in emission intensity has been ascribed to the binding of **1** in the hydrophobic pocket of serum albumin. The binding constants of **1** with BSA and egg albumin were calculated to be $(6.75 \pm 0.1) \times 10^4$ and $(5.4 \pm 0.1) \times 10^4$ M^{-1}, respectively, from the Scatchard plot, and the binding site size number was estimated to be one. Binding of complex **1** to lysozyme, however, resulted in only a marginal increase in its emission intensity. This is not surprising since lysozyme has a less hydrophobic region than the albumins. To understand the effect of albumin on the lifetime of the excited state of complex **1**, emission decay of the complex was monitored in the presence and absence of BSA (Figure 3). From the emission decay profile, the excited-state lifetime of the complex was estimated to be 1.89 μs in the presence of BSA.

Unlike in the case of BSA and lysozyme, the emission intensity of complex **1** decreases on addition of increasing

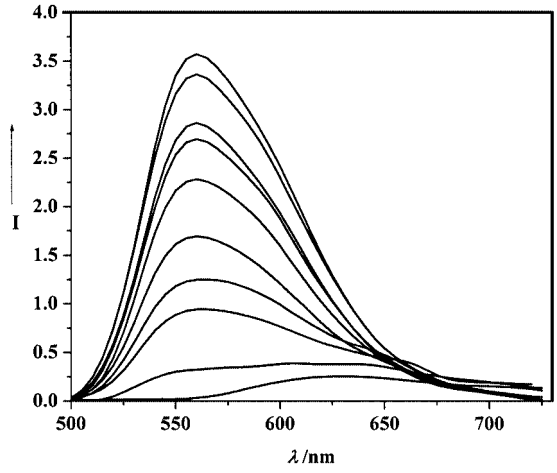

Figure 2. Emission spectra of **1** (10 μM) in the presence of BSA (0–5 μM) (λ_{ex} = 370 nm; pH 7.0).

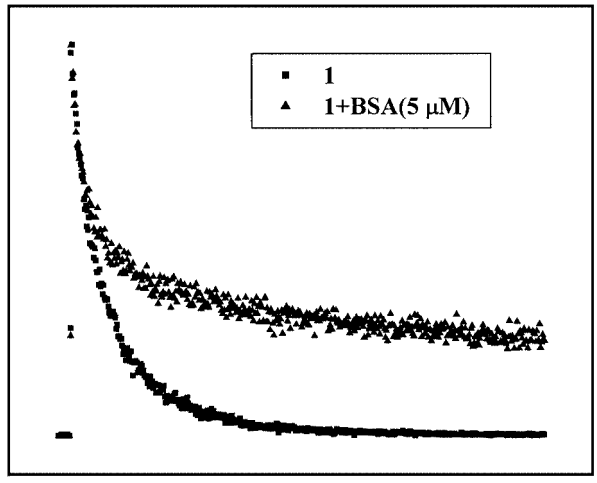

Figure 3. Decay profile of **1** (5 μM) in the absence and presence of BSA (5 μM) (298 K; pH 7.0).

amounts of hemoglobin (Figure 4). In hemoglobin protein, the hydrophobic pocket is occupied by the heme. If complex **1** binds to hemoglobin near the vicinity of heme, one can expect fluorescence resonance energy transfer (FRET) from complex **1** to the heme. This phenomenon of FRET will lead to quenching of the emission of **1** upon binding to hemoglobin. A careful examination of the crystal structure of hemoglobin shows that two sulfur-containing amino acids Met32 and Cys104 in subunit C are at a distance of 0.9 and 1.3 nm from the hydrophobic region where heme is present. Hence, binding of platinum(II) complex to these two sulfur sites will place complex **1** at the appropriate distance from heme for fluorescence energy transfer from **1** to the heme. As platinum(II) has a high affinity for sulfur,[12] complex **1** can be expected to bind to one of these two sulfur-containing amino acids of subunit C, resulting in the observed phenomenon of resonance energy transfer to heme.

In order to support the binding of complex **1** to the sulfur residues, we carried out the absorption titration of com-

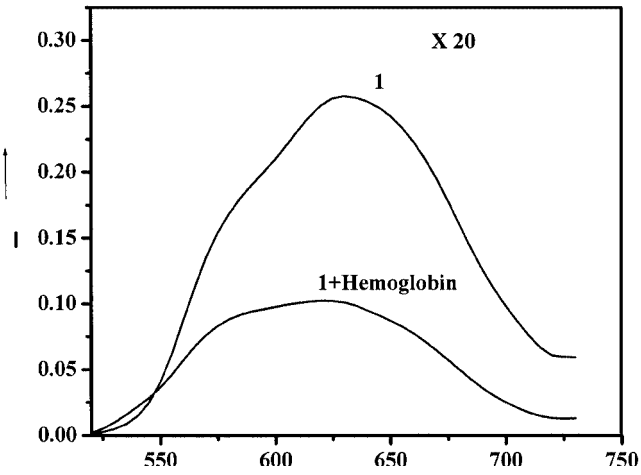

Figure 4. Emission spectra of **1** (50 μM) in the absence and presence of hemoglobin (5 μM) (λ_{ex} = 370 nm; 298 K, pH 7.0).

plex **1** with L-cysteine and L-methionine. Upon addition of increasing amounts of L-cysteine from 0 to 5 μM, the MLCT band of complex **1** shifted towards lower wavelength with the appearance of a new absorption band at 349 nm (Figure 5). The binding constant was determined from the following equation

$$[AA]/(\varepsilon_a - \varepsilon_f) = [AA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

where [AA] is the concentration of amino acid and ε_a, ε_f, and ε_b correspond to A_{obsd}/[Pt], the molar extinction coefficient of the free platinum(II) complex, and the extinction coefficient for the platinum(II) complex in the fully bound form, respectively. A plot of [AA] vs. [AA]/($\varepsilon_a - \varepsilon_f$) gives K_b as the ratio of the slope to the intercept. The binding constant (K_b) was found to be $(3.3 \pm 0.2) \times 10^5$ M^{-1}. However, in the presence of L-methionine, there is no change in the absorption spectrum of complex **1**. The spectral titration

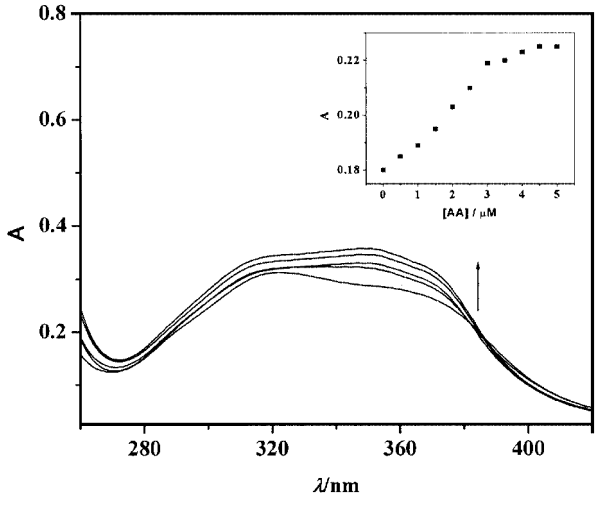

Figure 5. Absorption spectra of complex **1** (5 μM) in the absence and presence of L-cysteine (0–5 μM). Inset: plot of [AA] vs. absorbance at 349 nm.

studies reveal that [Pt(bzimpy)Cl]$^+$ binds more strongly to L-cysteine than to L-methionine. Hence, it can be concluded that complex **1** binds to Cys104 in the subunit C of hemoglobin.

To conclusively establish that complex **1** indeed binds to the hydrophobic regions of the protein, urea-unfolding experiments were carried out. Initially, the emission intensity of **1**-BSA adducts increases significantly with an increase in urea concentration and reaches a maximum at a urea concentration of 4.5 M. No further change in the emission intensity of the **1**-BSA adduct is observed with an increase in urea concentration up to 5 M. At 6 M urea, the emission intensity is the same as that of the native **1**-BSA adduct in the absence of urea. Beyond 6 M urea, however, there is a drastic decrease in the emission intensity of the adduct (Figure 6). Since BSA is a polypeptide with three domains (I, II, and III) extended through helices, the initial increase in emission intensity of the **1**-BSA adduct could be due to tight helical twisting in domain II of the BSA structure. The lack of any decrease in the emission intensity of the adduct in the presence of 4.5–5 M urea is indicative of the retention of the native-like conformation of domain II at this urea concentration. The marked decrease in emission intensity beyond 6 M urea and complete quenching beyond 7 M urea reveals the unfolding of the polypeptide chains followed by separation of domains from each other, which results in loss of hydrophobicity. The results observed here are similar to those observed for bilirubin binding to domain II of the BSA structure.[13] These results confirm that the binding of complex **1** to BSA is through the domain II hydrophobic cavity.

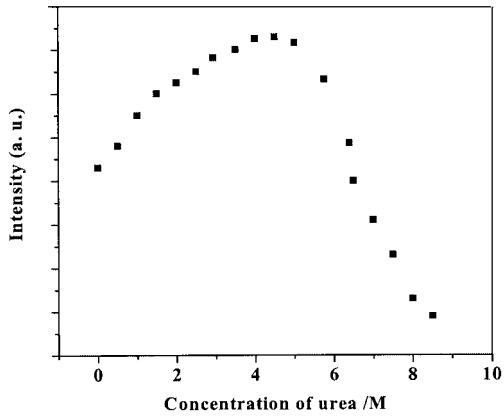

Figure 6. Unfolding of BSA incubated with **1** by urea (measured at 553 nm; λ_{ex} = 370 nm; 298 K).

In summary, we have shown that the present platinum(II) complex can be used as an extrinsic fluorophore for protein-binding studies. We hope that our findings will facilitate a significant development of metal-based sensors for biological applications. Further work is going on to study the uptake of this platinum(II) complex and its distribution in the cell, which can be monitored by fluorescence microscopy without addition of any external fluorophore.

Experimental Section

Materials: BSA (fraction V powder, lipid free, 66 kDa) and potassium tetrachloroplatinate(II) (K_2PtCl_4) were purchased from Sigma (St. Louis, MO, USA) and used as such without any further purification. 2,6-Pyridinedicarboxylic acid and *o*-phenylenediamine were purchased from Lancaster Chemicals. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) was purchased from SRL Chemicals. All other reagents were of analytical grade. All the experiments were carried out at pH 7.0 at 25 °C.

Synthesis of [Pt(bzimpy)Cl]PF_6 (1): The ligand bzimpy was prepared as reported in the literature.[14] The platinum(II) complex was prepared by refluxing K_2PtCl_4 (0.48 g, 1 mmol) in DMSO for 2 h followed by the addition of bzimpy (0.311 g, 1 mmol). The resultant yellow precipitate was filtered and dried with diethyl ether. The yellow precipitate was dissolved in acetone and precipitated as its PF_6^- salt by addition of a saturated NH_4PF_6 solution. The yellow precipitate turns red on drying. Yield: 0.6 g (80%). MS: *m*/*z* = 541.5 $[M - Cl]^+$. $C_{19}H_{13}ClF_6N_5PPt$: calcd. C 33.23, H 1.91, N 10.2; found C 32.91, H 1.85, N 10.12. ^1H NMR [(D_6)DMSO]: δ = 8.32 (m, 1 H), 8.05 (d, 2 H), 8.02 (d, 2 H), 7.62 (d, 2 H), 7.33 (t, 2 H), 7.29 (t, 2 H) ppm.

Studies on the Interaction of the Platinum(II) Complex with BSA

Spectroscopic Measurements: All absorption spectra were measured with a Perkin–Elmer Lambda 35 double-beam spectrophotometer. To understand the binding of L-cysteine and L-methionine to complex **1**, the absorption spectra of complex **1** (5 μM) was monitored as a function of concentration of the two amino acids (0–5 μM). The concentration of L-cysteine and L-methionine was varied until there was no appreciable change in the spectrum of the metal complex.

Emission Studies: Steady-state emission studies of the platinum(II) complexes were carried out by varying the concentration of BSA ($0–5 \times 10^{-6}$ M) while keeping the concentration of metal complex constant. The metal complex was excited at 375 nm and emission was monitored between 500 and 850 nm. The binding constant of complex **1** with BSA was determined from the Scatchard plot.[15]

Time-resolved emission for the complex was determined with a picosecond-laser-excited TCSPC spectrometer. The excitation source was a tunable Ti-Sapphire laser (Tsunami Spectrophysics, USA) with a pulse width of less than 2 ps and a repetition rate of 82 MHz. Samples were excited at 370 nm and the emission was monitored with an MCP-PMT (Hamamatsu-C 4878) detector. The decay traces were deconvulated by using a non-linear least-squares analysis with IBH software.

Acknowledgments

V. G. V. thanks the CSIR for a research fellowship. The authors thank the National Center for Ultrafast Processes, Taramani, Chennai, for providing the facilities to carry out time-resolved emission experiments.

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Received: April 21, 2005
Published Online: August 10, 2005

