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Water-Soluble and Biocompatible Cyclometalated Iridium(III) Complexes: Synthesis, Luminescence and Sensing Application

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Two new iridium(III) complexes with good water-solubility and biocompatibility have been synthesized and characterized. These two iridium(III) complexes showed strong luminescence with high quantum efficiency. Preliminary experiments demonstrated the feasibility of sensing lectin by formation of iridium(III) complexes. These new iridium(III) complexes might open up new applications in biosensor and chemosensor.

Introduction

Recently, iridium(III) complexes[1] have attracted more and more attention due to their relatively long excited-state lifetime, high photoluminescence efficiency, and excellent color tuning. The high quantum yield and long lifetime properties of iridium(III) complexes make them potentially useful in applications such as solar energy conversion, molecular sensing, and photocatalysis.[2] The ability to tune the luminescence wavelength in these systems also makes them useful for electrochemiluminescence (ECL) detection,[3] and various derivatives of *ortho*-metalated iridium(III) complexes with fluorinated aromatic ligands[4] and different substituents on the chelating ligands are also demonstrated for chemosensing in subsequent studies.[5] However, the synthesis of iridium(III) complexes with good water-solubility have not been achieved till now, and all previous studies on iridium(III) complexes were carried out in organic solvents. To facilitate the application of these luminescent materials to a wider diversity and scope, modification of complexes by appending carbohydrates on the chelating ligand was attempted. Addition of a carbohydrate to the polypyridine scaffold provided advantages of reducing the toxicity and improving the water solubility, and opens up the possibility of molecular targeting of carbohydrate-binding domains in cells and tissues.[6] In this communication, water-soluble and biocompatible iridium(III) complexes with appended sugar have been synthesized and characterized, and their feasibility of sensing lectin was also investigated.

Results and Discussion

4,4′-(Bromomethyl)-2,2′-bipyridine was synthesized according to a literature method with some modifications.[7] Functionalized polypyridine ligand was achieved by reacting 4,4′-(bromomethyl)-2,2′-bipyridine with commercial 1-thio-β-D-glucosetetraacetate by a modified literature procedure, then the acetyl esters was cleaved under basic conditions with sodium methoxide in methanol to give the unprotected ligands as white solids. The ^{1}H NMR and ^{13}C NMR spectroscopic data were identical to the literature.[8] The dichloro-bridged dimer $[Ir(CN)_2\text{-}\mu\text{-}Cl]_2$ was conveniently prepared from a reaction of the respective ligand and $IrCl_3{\cdot}xH_2O$.[1a] The functionalized polypyridine ligand was then reacted with $[Ir(CN)_2\text{-}\mu\text{-}Cl]_2$ to give the target complexes **1** and **2** (Scheme 1) as yellow and orange-yellow solids, respectively. The complexes were easily purified by filtering off the non-soluble sugar-substituted bipyridine ligands. The identities of the ligands and the complexes were confirmed by ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy, elemental analysis and ESI mass spectrometry. Comparison with the free ligand in the ^{1}H and ^{13}C NMR spectra, one isomer was formed in the complex **1** and two isomers were formed in the complex **2** probably due to the large structure hindrance of CN ligand. The chemical shift signal at δ = 8.43 and 8.37 ppm corresponding to H3-bpy in complex **2** evidently showed that the ratio of the two isomers was about 1:1.

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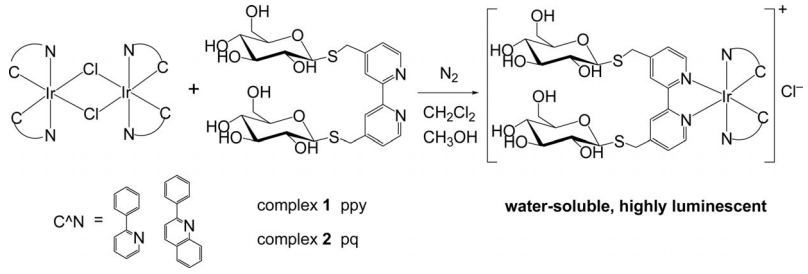

Scheme 1. Synthesis of water-soluble iridium(III) complexes.

Both of the newly synthesized iridium(III) complexes **1** and **2** are highly soluble in water. The photophysical data of the iridium(III) complexes in water were summarized in Table 1. The electronic absorption spectra of complexes **1** and **2** in water were mainly dominated by intense high-energy absorption bands at ca. 255–319 nm, and a comparatively less intense low-energy band at ca. 336–465 nm, which tailed off to ca. 510–540 nm. With reference to previous spectroscopic studies on related cyclometalated iridium(III) diimine systems,[9] these absorption bands with extinction coefficients in the order of 10^4 $dm^3 mol^{-1} cm^{-1}$ were ascribed to spin-allowed $\pi \rightarrow \pi^*$ intraligand (^{1}IL) transitions of the cyclometalating ligands or substituted bpy ligand. The less intense low-energy absorption bands at ca. 336–465 nm were likely to be originated from $d\pi(Ir) \rightarrow \pi^*$(ligand) spin-allowed metal-to-ligand charge-transfer (^{1}MLCT) transition. Similar assignments for the related cyclometalated iridium(III) diimine systems were also reported in the literature.[9] The weak absorption tails at ca. 510–540 nm was tentatively assigned to spin-forbidden ^{3}MLCT $d\pi(Ir) \rightarrow \pi^*$(ligand) transition, characteristic of the cyclometalated iridium(III) diimine systems.[9] There were no obvious changes in the electronic absorption spectra of the newly synthesized iridium(III) complexes in water stored in the dark for 24 h, demonstrating their good stabilities in aqueous solution.

Table 1. Photophysical properties of the iridium(III) complexes **1** and **2** at room temperature.

	λ_{abs} /nm (ε /$10^4$$dm^3$ mol^{-1} cm^{-1})	λ_{em} /nm	ϕ_{em}	τ /μs
1	255 (3.15), 318 (1.01), 379 (0.39), 465 (0.064)	582	0.26 (0.35)	0.19 (0.05)
2	266 (4.61), 319 (1.81), 336 (1.38), 432 (0.34)	557	2.84 (3.09)	1.02 (0.53)

The data of complexes were measured in water at 298 K. The excitation wavelength for complexes **1** and **2** was 371 and 430 nm, respectively. The quantum yields were calculated using $[Ru(bpy)_3]Cl_2$ in aqueous solution as the standard ($\phi_{Ru}^{II} = 1.0$). The data in parentheses were measured in aerated aqueous solution.

Complexes **1** and **2** were found to emit strongly at room temperature upon excitation with both UV and visible light in water solution with emission maxima at ca. 582 and 557 nm, respectively. With references to previous spectroscopic studies on other related cyclometalated iridium(III) diimine systems,[9] the strong emission was tentatively assigned to a spin-forbidden $d\pi(Ir) \rightarrow$ *(ligand) metal-to-ligand charge-transfer (^{3}MLCT) excited state, probably with some mixing of a spin-forbidden $\pi \rightarrow \pi^*$ intraligand (^{3}IL) character. It was interesting to find that the luminescence quantum efficiency of complex **2** in water was much higher than $[Ru(bpy)_3]Cl_2$ ($\phi_{Ru}^{II} = 1.0$), which would make it more sensitive in the analytical and bioanalytical applications.

Cell viability upon treatment with the new iridium(III) complexes **1** and **2** was determined by testing the mitochondrial enzyme function according to the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, since living cells are capable of reducing light colored tetrazolium salts into intensely colored formazan derivatives.[10] Figure 1 shows the viability of HepG2 cells upon treatment with the new iridium(III) complexes for 24 h. The results revealed that the new iridium(III) complexes exhibited very low cytotoxicity on HepG2 cells, where the treatment of HepG2 cells with a series of dilutions (5, 25, 50, 75 and 100 μM) of the new iridium(III) complexes resulted in a slight decrease in cell viability. Even at the highest dosage (100 μM), complex **1** and **2** only decreased the cell viability by 2% and 6% in 24 h, respectively. We have also examined healthy HepG2 cells treated with 100 μM of new iridium(III) complexes (30 min incubation at 37 °C in phosphate buffer) by confocal microscopy. Morphologically intact cells were not

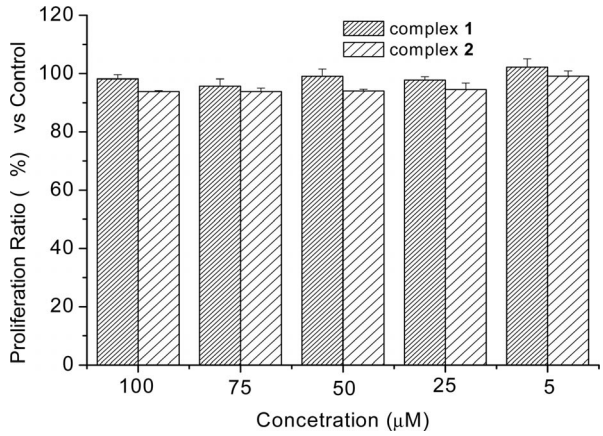

Figure 1. The viability of HepG2 cells upon treatment with the new Ir^{III} complexes for 24 h.

stained, which suggested that the cell membrane was impermeable to iridium(III) complexes, which is consistent with previous reports that the sugar-substituted complexes should not be able to be internalized by intact cells at all.[11]

Viologens, the cationic salts of 4,4′-bipyridinium, are well-known electron acceptors that have been found to quench the fluorescence of numerous dyes[12] and macromolecular systems.[13] Since the boronic acid derivative of methylviologen (MV^{2+}) has a high affinity for sugar, *N*,*N*′-bis(benzyl-3-boronic acid)-4,4′-bipyridinium dibromide (m-BBV) has been used extensively in the development of sugar sensors. Figure 2 shows the luminescence changes of the iridium(III) complex **2** upon addition of BBV. Upon addition of BBV to a water solution of iridium(III) complex **2**, the luminescence of the complexes was found to decrease in intensity with a small blue shift. The luminescence intensity of complex **2** was significantly quenched by the addition of the BBV (500 equiv.) with a reduction of ca. 85% in the intensity, which might be ascribed to the binding of the BBV to the sugar. The quenching of the luminescence was probably due to the electron transfer from the iridium(III) complex to BBV.[14]

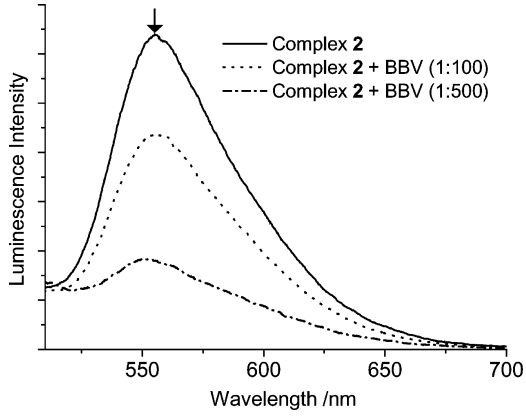

Figure 2. Luminescence changes of the Ir^{III} complex **2** (0.5×10^{-5} mol/L) in aqueous solution upon addition of various amounts of BBV.

Lectins are carbohydrate-binding proteins that mediate important biological processes such as cell growth, the inflammatory response and viral infections.[15] Currently there is a great interest in lectin detection using sugar-substituted luminescent metal complexes.[14,16] Concanavaline A (Con A) was selected as a target to study the capability of complex **2** as lectin sensor. The emission properties of the iridium(III) complex **2** upon addition of Con A were investigated and shown in Figure 3. The luminescence intensity of iridium(III) complex **2**/BBV was increased upon addition of the Con A. The strong binding affinity between glucose-iridium(III) complex and the lectin probably result in separating the quencher (BBV) from the iridium(III) complex, and thus increasing the luminescent signal. These results confirmed that the water-soluble iridium(III) complex **2** could act as a lectin biosensor.

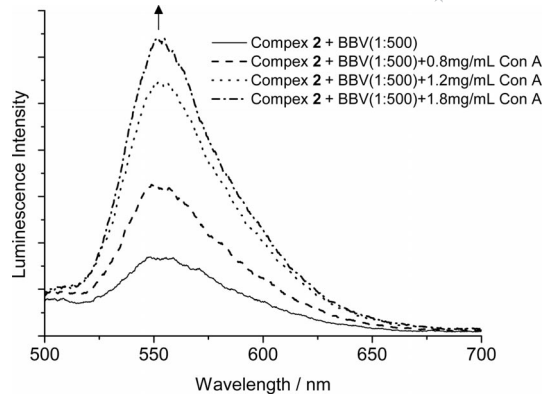

Figure 3. Luminescence changes of the Ir^{III} complex **2**/BBV system (complex **2** 5.0×10^{-6} mol/L, BBV 2.5×10^{-3} mol/L) upon addition of Con A. Measured in 0.01 mol/L phosphate buffer (containing 0.1 mmol/L Ca^{2+}, Mn^{2+}).

Conclusions

In conclusion, two new iridium(III) complexes with good water-solubility and biocompatibility have been synthesized and characterized. These two iridium(III) complexes showed strong luminescence with high quantum efficiency. Preliminary experiments demonstrated the feasibility of sensing lectin with iridium(III) complexes. This work also provided a strategy for other luminescent complexes for the biological sensing. Further investigation on conditioning iridium(III) complexes as ECL-based biosensors is still being carried out in our lab.

Experimental Section

General: MTT assay was used to determine viability of HepG2 cells upon treatment with the new iridium(III) complexes **1** and **2**, as described in detail elsewhere.[10] HepG2 cells were seeded in 96-well tissue culture plates at the density of 4×10^6 cells per well and incubated for 3 d. After the treatment with the new iridium(III) complexes for 24 h, the plates were washed twice with culture medium, and then MTT was added and incubated for another 4 h. Cells without treatment of the new iridium(III) complexes were used in a control experiment. The relative cytotoxicity was expressed as percentage of $[OD_{sample} - OD_{blank}]/[OD_{control} - OD_{blank}] \times 100$ (OD, optical density). Each experiment was performed in triplicate.

Supporting Information (see footnote on the first page of this article): Synthetic procedures, characterization of complexes, NMR spectra and ESI spectra.

Acknowledgments

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